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(June 1, 1993-September, 1994)  
(Sections I and II)

ON

*DESIGN AND PROCESSING  
OF MATERIALS  
BY BIOMIMICKING*

(AFOSR-91-0281)  
SECTION - I: RESEARCH PROGRESS

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## Section - I

(Section- II includes Appendices)

# *DESIGN AND PROCESSING OF MATERIALS BY BIOMIMICKING*

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## 1. Summary

This is the third years' Report for the 3-year grant entitled "Design and Processing of Materials by Biomimicking," monitored under the Grant # AFOSR-91-0281 covering the period June 1, 1993 through May 30, 1994. This report contains major research results of the first two years as well for completeness. The present project is now in its no-cost extension period that will end on March 1, 1995 (at which time we plan to present the latests results included to this one). The reports contains, in addition to the last two years, the latest accomplishments in various areas proposed for this period of the project; it also includes future directions (for the remaining no-cost extension period and beyond).

The research activities, undertaken by our collaborative groups in Materials Science and Engineering and Microbiology Departments (Fig. 1) are divided into five categories. These are: (i) selection and biological production of biopolymers for use in ceramic processing, (ii) processing of ceramic with biopolymers; (iii) A Comparison of Synthetic and Biopolymers for Safety Concerns; (iv) Mechanical property-microstructure correlations in biological composites (nacre of abalone and pinctada); (v) Ultrafine magnetic particle formation in bacteria, and (vi) Investigation of ultrastructure in biological hard tissue, nacre. The overall objective of the research is in all these areas is to learn lessons from biological systems to develop new strategies in the design and processing of novel synthetic materials. The research accomplished to date will be highlighted in the following sections.

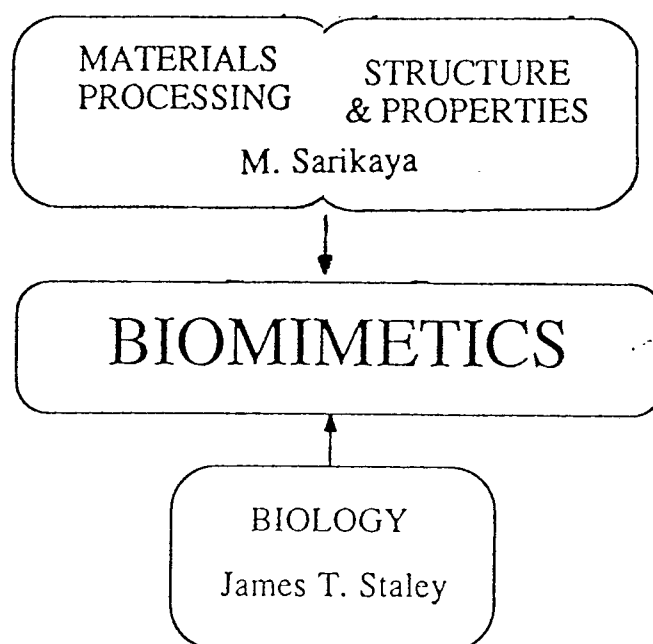


Figure 1 - Collaborative groups in Biomimetic Research

## 2.0 Brief Description of Research Accomplishments and Future Research

### 2.1 Use of Biopolymers in Ceramic Processing

#### 2.1.1. Selection and Biological Production of Biopolymers: Bacterial Polyglutamic Acid as Aid in Ceramic Processing

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#### i. Summary

The goal of this research has been to find dispersants for ceramic processing applications. The polyelectrolytes currently in use in the industry are synthesized from toxic precursors which present health hazards to workers. We have investigated polyelectrolytes produced from natural sources such as bacteria and kelp, as well as some polymers which have been produced synthetically.

The polyelectrolytes which we have investigated include polysaccharides (alginate and dextran) and polypeptides (polyglutamic acid and polyaspartic acid). Some have been obtained from cultures of bacteria grown in our laboratories, others have been obtained from chemical supply houses.

In addition, we have investigated the feasibility of using an *in situ* process by which we incubate the polymer-producing bacteria in the presence of ceramic powders. In this system, polymer adsorbs to the powder in a high affinity state, allowing subsequent washing to remove the bacterial cells, while retaining the adsorbed polymer.

During our *in situ* investigations we also made the fortuitous discovery that citric acid, a low molecular weight organic acid, also acts to stabilize the alumina/aqueous system. We characterized this system by testing some closely related organic acids containing (i) varying numbers of carboxylic acid groups and/or (ii) containing varying numbers of hydroxyl groups. As a result of this work (reported in Section 2.2) we have a better understanding of the mechanisms of adsorption.

## ii. Recent Results

We found that chemically synthesized polyaspartic acid and polyglutamic acid (PGA) can be used as dispersants for nanosized ceramic particles (AKP-30) in aqueous suspensions (Table-I). Packing densities of better than 50% theoretical have been achieved (Ren et al, 1992).<sup>1</sup>

A naturally produced polyglutamic acid was purified from cultures of *Bacillus licheniformis*. It also was effective as a dispersant but only in low solids loaded suspensions. In high solids loaded suspensions the viscosities were unacceptably high, apparently due to the high molecular weight of the polymer (Ren et al., in preparation).

We obtained lower weight natural PGA by partial acid hydrolysis of *B. licheniformis* PGA, followed by ultrafiltration to obtain a fraction with molecular weight between 1,000 and 10,000 daltons. This fraction was used to prepare 40 vol% solids loaded suspensions having a viscosity of only 112 cPs. (Table-II).

## iii. Objective and Justification of Research

The objective of the research is to investigate the possibilities of colloidal processing of ceramics by using biopolymers. In usual ceramic processing practice, polymers or surfactants are used to provide lubrication and plastic formation to achieve high density packing of ceramic particles. The currently used polymers in ceramic industry providing these properties, however, are harmful, expensive, and, therefore, their use is limited. Our aim, in this research, is to explore new polymers with biological origin that provide lubrication and, at the same time, lower viscosity of the ceramic suspensions beyond those can be achieved by synthetic polymers. Furthermore, biopolymers would economically be more desirable and environmentally sound. Our further aim is to use bacteria that would produce these biopolymers in the presence of ceramic suspensions and provide *in-situ* processing of ceramic materials.

## iv. Background

High technology ceramic parts, such as those used for automobiles, aircraft, superconductors, etc., need highly dense (nonporous) and fine-grained microstructures after sintering at relatively low temperatures. The ceramic particles (e.g., alumina) used for such applications are of very small diameter (400 nm) and, because of this small size, are subject to van der

Waals forces which cause the particles to aggregate. In order to achieve a dense and uniformly consolidated compact, the first requirement is to process dispersed colloidal suspensions which contain upwards of 50 vol% solids while still having relatively low viscosities of less than 1,000 mPas. The second key requirement is that these compacts must be plastically shaped into a net shape with the aid of lubricating surfactants.<sup>2,3</sup>

As a solution for the first requirement, particles are generally coated with surfactants to achieve colloidal stability. Our work during the 88-91 period of AFOSR supported research has shown that biopolymers function as well as the synthetically produced surfactants as dispersants.<sup>7</sup> With respect to the second requirement, however, we now expect that biopolymers or monomers to surpass their synthetic counterparts.

Since prehistoric times, potters have aged clay beds by amending them with organic materials such as tannins or urine. Early in this century, Stover<sup>4</sup> and Glick<sup>5</sup> and co-workers demonstrated that bacterial growth in clay beds increased the plasticity of the clays. We have previously reported that the acidic microbial polysaccharide (alginate) could aid in ceramic processing by coating nanosized alumina particles thereby allowing their close packing (Pellerin et al., 1992,<sup>7</sup> Ren et al., 1992<sup>6</sup>). In this study, we report that another acidic bacterial (also synthesized) polypeptide, namely polyglutamic acid (PGA), also serves as a dispersant for ceramic particles (Ren et al.,<sup>1</sup> 1992a and Ren et al., in preparation<sup>8</sup>). The work on lubricating properties will follow.

## **v. Results: Biopolymers as Dispersants for Ceramic Particles**

A. Materials: Two biopolymers were chosen in this period of the research. These are:

### **a. Alginate:**

This is prepared from either bacterial or algal kelp as the source or produced by cultures of *Azotobacter vinelandii*. The structure is a polysaccharide composed of mannuronic (Poly M) and guluronic acids (Poly G) in block repeating unites, -M-M-M-M-, -G-G-G-G.<sup>1</sup>

### **b. Polyglutamic Acid (PGA)**

The source is synthetic (Sigma) Chemical Co. or produced by cultures of *Bacillus licheniformis*. The structures is a polypeptide composed of an acidic amino acid, glutamic acid.<sup>1</sup>

Both polymers contain one free carboxylic acid group per residue.



## B. Procedures and Tests of dispersant qualities of the purified polymers

- Sedimentation packing density % of theoretical maximum with 2 vol% suspensions. Better dispersion of ceramic particles produce a higher packing densities in the green body.
- Viscosity measurements of 30, 40, and 50 vol% suspensions (high solid loading suspensions). In this case, better dispersion of ceramic particles means lower viscosities.

Ceramic powder: High purity (99.99%)  $\text{Al}_2\text{O}_3$ , with an average particle size (diameter) of 400 nm and a density of 3.96g/cc (AKP-30, Sumitomo Chemical America, Inc., New York, NY.).

Strain: *Bacillus licheniformis* ATCC 9945A

Polyglutamic acid harvest: The bacteria were agitated in modified medium E at 37C for 4-5 days. The cells were removed by centrifugation at 22,000 x g for 50 min. Three volumes of the -20C ethanol were then added to the supernatant for precipitation. The precipitate was washed with ethanol 2-4 times, and then dissolved in distilled water before dialyzing against distilled water for 48 hours. After dialysis the polyglutamic acid was lyophilized.

Sedimentation tests: Sedimentation columns were prepared by adding 2 vol% AKP-30 powder to aqueous solution with various concentrations of the polymer. The suspensions were sonicated for 5 minutes and mixed on a magnetic stirrer for 0.5-2 h. The pH of the suspension was adjusted to the experimental value before bringing the final volume to 10 ml. The suspension was decanted into a conical bottom, graduated polystyrene tube and left undisturbed at room temperature for 3-4 weeks.

Viscosity measurements: Viscosity measurements were obtained using a Rheometrics Fluid Spectrometer, Model 8400 (Rheometrics Inc., Piscataway, NJ.). Suspension (about 15 ml) for viscosity measurements were prepared as for the sedimentation tests.

Hydrolysis of the bacterial PGA: The purified bacterial polyglutamic was hydrolyzed by 3 N HCl for 10 min. at 100C, cooled, and then neutralized by NaOH. The hydrolyzed solution

was ultrafiltered through a MW 10,000 membrane first and the filtrate was collected. The filtrate was ultrafiltered through a MW 1,000 membrane and the remnant was washed 5-8 times by ultrafiltration to remove the NaCl salt from the neutralization before lyophilizing.

### C. Experimental Results

Packing densities in the wet cakes were, in some cases, better than 50% of the theoretical maximum; densities of 40% and better were easily achieved (Table-I).

Viscosity measurements of high solids-loaded suspensions show that these polymers are very effective in reducing intraparticle attractions (Table-II).

## vi. Conclusions

The results can be summarized as follows:

- Chemically synthesized polyaspartic acid and polyglutamic acid can be used as dispersants for nanosized ceramic particles (AKP-30) according to the rheological behavior of the aqueous suspension system (packing density and viscosity).
- A similar polyglutamic acid isolated and purified from *Bacillus licheniformis* has the same dispersion function.
- Partially hydrolyzed PGA with lower MW works better as a dispersant than the intact PGA with longer chain and this gives a further evidence that molecular weight is an important factor to effect the dispersion behavior of polymer.
- Therefore this biopeptide could be another bio-source or bio-system for the dispersion of nanosized ceramic powders that can be incorporated in to the current industrial practices.

The polypeptides chosen for this work contain only carbon, oxygen, nitrogen and hydrogen, all of which should be safely removed during pyrolysis, leaving no undesirable residues in the finished product.

## vii. Future work

Studies to date have focused on the identification of biopolymers that can act as dispersants for alumina particles. Two acidic polymers, alginate and polyglutamic acid, have been

identified and studied. More recent work involves the study of monomer acids including citric acid (Appendix 1), which also serves as an excellent dispersant.

Studies will be continued to identify polymers that might serve as dispersants as well as gelling agents for nanosized alumina particles. A variety of bacteria that produce polymers will be examined. The work is outlined below:

- o- Identification of polymer-producing bacteria,
- o- Purification of polymers,
- o- Testing of polymers for dispersant activity (sedimentation tests),
- o- Testing of polymers for gelling capability,
- o- Chemical characterization of polymers that show dispersant or gelling capability.

**Table-I: Packing densities (% theoretical) of AKP-30 alumina suspensions with polypeptides**

The 2 vol% columns were prepared with 0.792 g alumina in 8 ml distilled water/polymer solution. Polymer concentrations are dry weight basis (dwb) of clay.

	Polymer Concentration (% dwb)	Packing Density (% theoretical)
Poly-D-glutamic acid (synthetic)	0.5	36
	1.0	44
	6.0	53
Poly-L-glutamic acid (synthetic)	0.5	38
	1.0	44
Poly-D-glutamic acid (bacterial)	1.0	41
Poly-D-aspartic acid (synthetic)	0.5	41
	1.0	44
	4.0	51
Poly-L-aspartic acid (synthetic)	0.5	40
	1.0	46
	4.0	53
Control (no polymer)	0	8

**Table-II: Viscosities (in cPs.) of the AKP-30 suspensions dispersed by polyglutamic acid from various sources**

Synthetic PGA has a molecular weight range of 5,000 - 10,000 daltons. Native bacterial PGA has a molecular weight in excess of 100,000 daltons. Hydrolyzed bacterial PGA was between 1,000 and 10,000 daltons.

	Polymer Concentration (% dwb)	Solids Loading (vol%)		
		30	40	50
Synthetic PGA	0.5	5 cPs.	105	1115-2195*
	1.0	135	135	**
	3.0	430	ND	**
Bacterial PGA	0.5	30	280	**
	1.0	115-230*	>500	**
Hydrolyzed PGA	0.5	<50	112	1500-2500*

#### v. References

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8. Ren et al., in preparation (1993).

## 2.1.2 Processing of Ceramics with Biopolymers

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### i. Objectives and Justification of Research

The objective of this research is to utilize biomolecules as ceramic processing aids for which organic polymers are traditionally used. These biomolecules are environmentally compatible. Incorporating their unique properties can also lead to novel forming methods for ceramics.

Our specific interest is to utilize biomolecules in aqueous injection molding of ceramics. Two approaches were taken. First, the thermal gelation behavior of agarose was used in combination with biomolecules which served as dispersants for aqueous suspension of alumina. High-density and uniform green bodies can be shaped by this method. The second approach is to search for "lubricating biomolecules" that can adsorb on the particle surface and endow plasticity (only observed in natural clays) to concentrated ceramic slurries

### ii. Background

#### A. Aqueous Injection Molding by Thermal Gelation

Aqueous injection molding was first proposed by a research group at Allied-Signal. In their patent, ceramic powders were first dispersed by PMAA in water, and then agarose was added to the suspension. The resulting slurry, kept at temperature above the gelation point, was injected into a mold and gelled to form complex shapes when cooled.

Some previous work related to aqueous injection molding were performed in this collaborative group. Cesarano and Aksay<sup>1,2</sup> found that highly-concentrated ceramic suspensions can not be prepared from the above approach. When the low-density gelled specimens were dried, the large amount of shrinkage would make the fabrication of large ceramic objects impossible. They concluded that this phenomenon was due to the phase separation of dispersant and gelling agent. To solve this problem, compatible dispersant/gelling agent combinations must be found. Another related research was done by Pellerin et al.<sup>3</sup> In their work, Poly-G (a purified biopolymer) was shown to be an effective dispersant for aqueous suspension of ceramics. Up to 50 vol% alumina suspensions could be prepared by

using poly-G as the dispersant. Recently, Ren et al.<sup>4</sup> found that citric acid could serve as a more effective dispersant for alumina in aqueous suspension. Alumina suspension with >55 vol% of solids can be prepared by using 0.5 wt% citric acid (this is included in Section 2.2).

Based on these previous studies, the approach taken in this research was, therefore, to use poly-G and citric acid as dispersants in combination with agarose. It is expected that the problem of phase separation can be solved and that aqueous injection molding be improved and practically utilized for forming ceramics.

Dispersant	Gelling Agent
Poly-G	Agarose
Citric Acid	

#### B. "Lubricating" Biomolecules

The unique plasticity of clays allows them to be shaped by different plastic forming methods, including injection molding, extrusion, and jiggering. However, for advanced ceramics, plasticity is only possible with low density cakes. The low-density green bodies formed by these techniques have to be sintered at higher temperatures. In addition, the linear shrinkage after firing is large and therefore plastic forming of advanced ceramics is only feasible for fabricating objects of small cross-sections.

Another objective of this research is, thus, to search for lubricating polymers that could possibly lead to plasticity of advanced ceramics. Based on the promise that there are an abundance of biomolecules which might be available adsorbing on clay surface, which might be the cause for clay plasticity, we started to search from biomolecules that could adsorb on the particle surface of ceramics and can lead to plasticity. Also, the salt-coagulation phenomenon, which was suggested by Lange et al.<sup>5</sup> to possibly lead to clay plasticity, was also studied in order to understand its mechanism.

### iii. Results

#### iii.a. Summary of Results of the 1st Period (May 1991 - June 1992)

##### i. Aqueous Injection Molding by Thermal Gelatin

##### a. Poly-G + Agarose

Poly-G stabilized 45 vol% alumina suspensions were prepared. Up to 1 wt% agarose can be added into the alumina suspensions without causing de-stabilization. These suspensions can be cast to form complex shapes without causing cracking during drying.

#### b. Citric Acid + Agarose

This combination was shown to be a better system. Up to 50 v/o alumina suspensions stabilized with citric acid and containing 0 -1 wt% agarose can be prepared. These highly concentrated suspensions have low viscosity and can be poured into molds, gelled to form complex shapes.

#### ii. "Lubricating" Biomolecules

The consolidation behavior of the salt-coagulated alumina suspensions was studied using centrifuge consolidation and gamma-ray densitometry. The existence of "hydration force" improves consolidation of these coagulated alumina suspensions. However, this short range "hydration force" does not endow plasticity to the consolidated cakes.

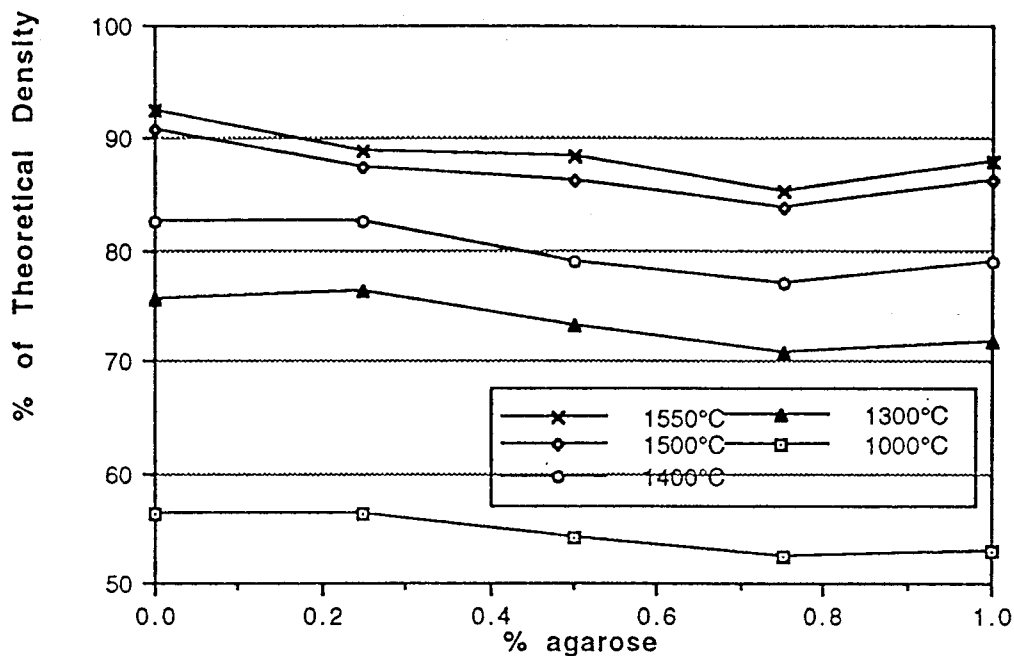
#### iii.b. Summary of the Results in the 2nd Period (May 1992 - June 1993)

##### i. Aqueous Injection Molding by Thermal Gelatin

Samples cast from 45 vol%  $\text{Al}_2\text{O}_3$ /Poly-G/Agarose and 50 vol%  $\text{Al}_2\text{O}_3$ /Citric Acid/Agarose suspensions were dried and sintered at 1000°C to 1550°C. The results of the sintering behavior are shown in Fig. 1 and 2, respectively. The sintered densities of those cast from the 45 vol%  $\text{Al}_2\text{O}_3$ /Poly-G/Agarose suspensions are relatively low. Even the one without agarose has a sintered density of only 92% at 1550°C. Those from the 50 vol%  $\text{Al}_2\text{O}_3$ /Citric acid/Agarose suspensions are higher. The one containing 0.8 wt% agarose can be sintered to 96% density at 1500°C. These results again indicate that citric acid is a better dispersant.

In general, the sintered density decreases as the amount of the agarose increases. This does not necessarily mean that the polysaccharide residues has adverse effect on sintering of alumina. An examination of the microstructure of the sintered samples shows that large amount of spherical pores are present, which are likely coming from air bubbles trapped in suspensions during the mixing of suspensions. Improvement in the mixing and de-airing treatment were thus made to solve this problem. Currently, 55 vol%  $\text{Al}_2\text{O}_3$ /Citric acid/Agarose suspension can be prepared and cast. Sintering experiments are undergoing to evaluate the improved processing method.

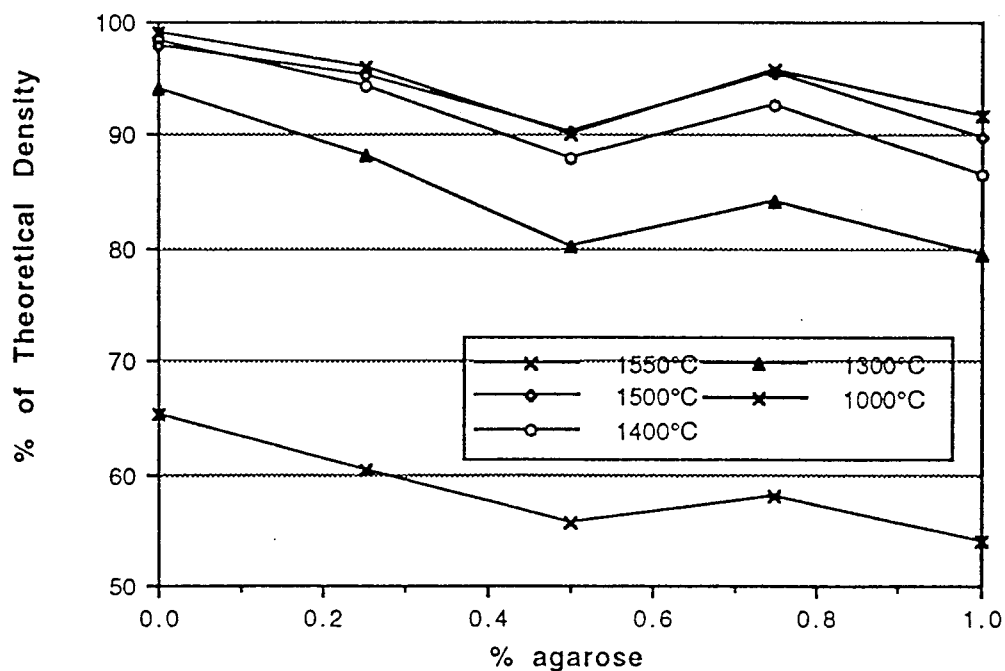
Sintered Density of Samples Cast from  
45 v/o AKP-30 stabilized with Poly-G



Data from "Total Sintered Densitv.CG2"

Figure 1

Sintered Density of Samples Cast from  
50 v/o AKP-30 stabilized with Citric Acid



Data from "Total Sintered Density.CG2"

Figure 2



## ii. "Lubricating" Biomolecules

It is considered that a combination of short-range repulsion and long-range attraction interparticle forces is required to create "lubrication" effect. In aqueous suspensions, the long-range attraction is already imparted by van der Waals force. It is planned to systematically change the range of steric repulsion by adsorption of small molecules, oligomers and block-copolymers and study the effect on consolidation behavior. Various 15 vol%  $\text{Al}_2\text{O}_3$  suspensions stabilized with citric acid and poly-G were prepared. The pH values of these suspensions were varied to change the magnitude of the electrostatic repulsion. These suspensions were centrifuged at different speed and the average density of the consolidated cakes were measured. Viscosities of the suspensions were also measured to give us an indication of the magnitude of interparticle forces.

The results for the 0.2 and 0.5 wt% citric acid stabilized suspensions are shown in Fig. 3, and 4, respectively. In general, when the viscosity of the suspensions reduces, indicating a reduction in the overall attraction forces, the density of the consolidated cakes increases. The magnitude of the overall interparticle attraction seems to be the dominating factor in determining the consolidation behavior. The effect of the short range repulsion is not clear from these results. More experimental work is needed to study these effects.

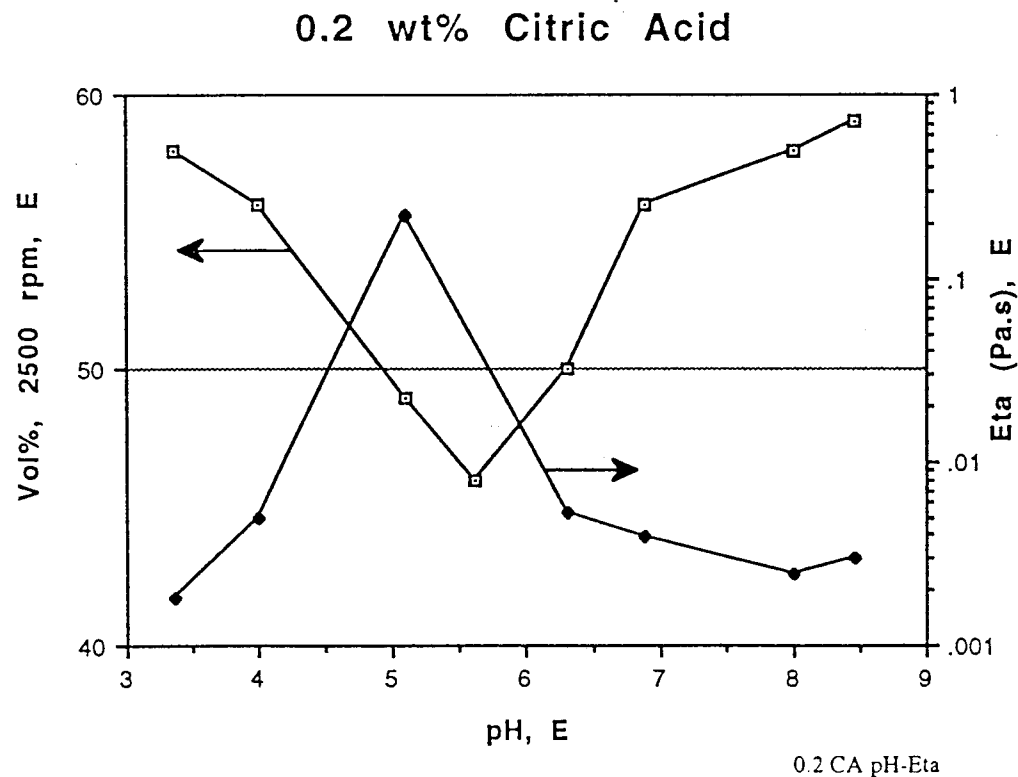


Figure 3

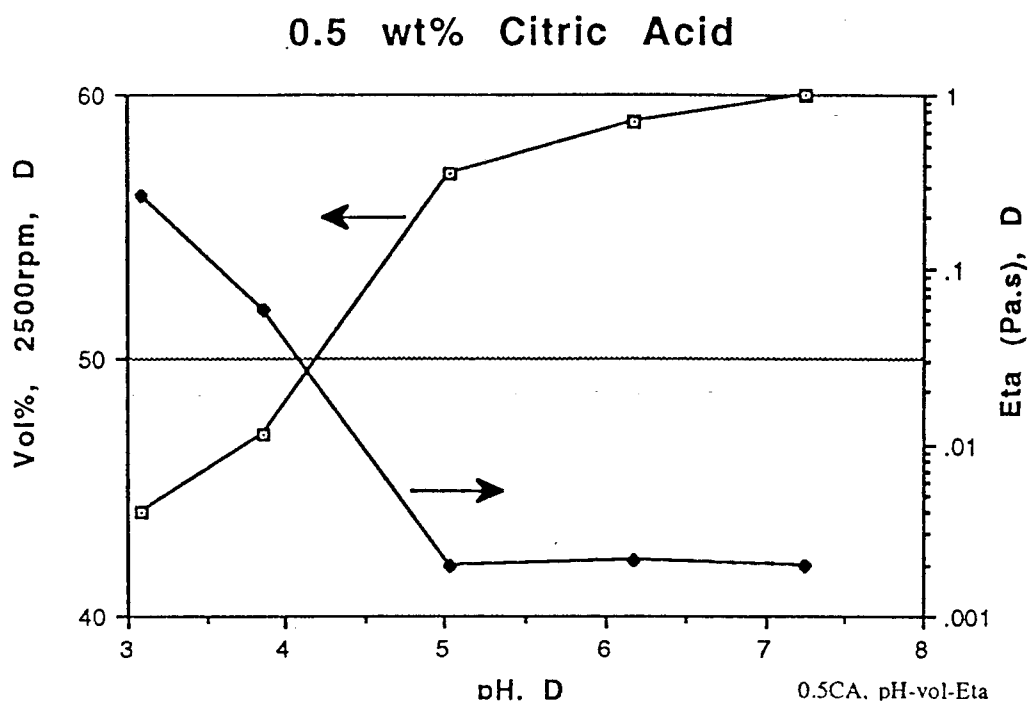


Figure 4

#### iv. Future Work

##### i. Aqueous Injection Molding by Thermal Gelation

The selected dispersant/gelling agent combinations are shown to be capable of preparing highly concentrated ceramic suspensions which could be used for aqueous injection molding. Those prepared from citric acid/agarose system can be used to prepare 55 vol% alumina suspensions and then gelled to form shapes. This is advantageous compared with the original PMAA/agarose system and the conventional injection molding in which organic polymers are used.

Now that the processing methods are optimized, we plan to characterize dispersant/gelling agent in terms of its phase compatibility and its effect on the consolidation, sintering behavior, and resulting microstructure of ceramics. The "competitive adsorption" of monomer and polymer also needs to be studied.

## ii. "Lubricating" Biomolecules

The study of the effect of systematically changing the range of steric repulsion on the consolidation behavior of weakly-flocculated alumina suspensions will be continued. Also, the effect of conformation of the adsorbed polymers will be examined.

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## 2.2. Hydroxylated Carboxylic Acid Monomers as Dispersants for Ceramic Particles

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### i. Objectives

The purpose of this investigation is to explore new biological dispersants in colloidal ceramic particle processing. The objective is to search for monomers, characterize their dispersion properties, and investigate mechanisms of packing in terms of properties and structures of the biological dispersants.

The justification for this study are due to: (i) the necessity of use of low density dispersants, such as biomonomers, for high density packing (lower the mol. wt. of polymer, higher the packing density), (ii) the ease of controlling dispersant with the gelling agent (to prevent premature gelling or phase separation), (iii) ease of biodegradability or environmental compatibility, and (iv) lower cost.

### ii. Summary of the Preliminary Research\*

Carboxylic Acids, which contain one to three negatively charged  $\text{--COO}^-$  group(s), were investigated as dispersion additives in the processing of ceramic particles. A total of 21 of these carboxylic acids were examined for dispersion by sedimentation tests in this study (See Table - I). Only carboxylic acids containing more than one hydroxyl group were able to disperse  $\text{Al}_2\text{O}_3$  particles (AKP-30 Sumitoma Co., 400 nm dia.) in aqueous suspensions.

The packing densities of the ceramic suspensions varied from 30-50% of the theoretical density depending on the number of hydroxyl groups and carboxylate groups. Four carboxylic acids, i.e., citric acid, tricarballic acid, meso tartaric acid, and fumaric acid, were studied in detail by measurement of zeta potential, particle packing, and dispersant absorption characteristics, to compare their dispersion behavior.

These detailed studies further indicate that carboxylic acid groups and hydroxyl groups are both important for dispersion of ceramic particles. Thus organic acids containing only the

Table 1: Carboxylic acids investigated in this study with their molecular components, acidic constants and solubilities.

Name	Structure	pKa	Solubility (g/100 ml H <sub>2</sub> O)
<b>Mono-carboxylic acids</b>			
Glutamic	$\text{HOOCCH}(\text{NH}_4^+)(\text{CH}_2)_2\text{COOH}$	2.19, 4.25, 9.67	80-200
Mannuronic	$\text{HOOC}[\text{CH}(\text{OH})]_4\text{CHO}$	-	-
<b>Di-carboxylic acids</b>			
Adipic	$\text{HOOC}(\text{CH}_2)_4\text{COOH}$	4.43, 4.41	2
Mucic	$\text{HOOC}[\text{CH}(\text{OH})]_4\text{COOH}$	-	-
Fumaric ( <i>trans</i> )	$\text{HOOCCH}=\text{CHCOOH}$	3.03, 4.44	0.7
<i>meso</i> -Tartaric	$\text{HOOCCH}(\text{OH})\text{CH}(\text{OH})\text{COOH}$	3.22, 4.82	167
Succinic	$\text{HOOC}(\text{CH}_2)_2\text{COOH}$	4.16, 5.61	6
Maleic ( <i>cis</i> )	$\text{HOOCCH}=\text{CHCOOH}$	1.83, 6.07	79
Malic	$\text{HOOCCH}_2\text{CH}(\text{OH})\text{COOH}$	3.40, 5.11	138
<i>d,l</i> -Tartaric	$\text{HOOC}[\text{CH}(\text{OH})]_2\text{COOH}$	2.98, 4.34	21
Malonic	$\text{HOOCCH}_2\text{COOH}$	2.83, 5.69	74
Tartronic	$\text{HOOCCH}(\text{OH})\text{COOH}$	2.42, 4.54	-
Mesoxalic	$\text{HOCCOCOOH}$	-	-
Glutaric	$\text{HOOC}(\text{CH}_2)_3\text{COOH}$	4.34, 5.41	64
Sebacic	$\text{HOOC}(\text{CH}_2)_8\text{COOH}$	4.6, 5.6	-
<b>Tri-carboxylic acids</b>			
Tricarballic	$\text{HOOCCH}_2\text{CH}(\text{COOH})\text{CH}_2\text{COOH}$	3.6, 4.7, 5.8	-
Aconitic ( <i>trans</i> )	$\text{HOOCCH}_2(\text{COOH})=\text{CHCOOH}$	2.8, 4.6, -	-
Nitrilotriacetic	$\text{HOOCCH}_2\text{N}(\text{CH}_2\text{COOH})\text{CH}_2\text{COOH}$	3.03, 3.07, 10.7	-
Citric	$\text{HOOCCH}_2\text{C}(\text{OH})(\text{COOH})\text{CH}_2\text{COOH}$	3.13, 4.76, 6.40	-
<b>Tetra-carboxylic acids</b>			
Ethylenediamine tetraacetate (EDTA)	$\text{HOOCCH}_2\text{N}(\text{CH}_2\text{COOH})\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{COOH})\text{CH}_2\text{COOH}$	-	-

Note: The data are from 1) --- Index, and 2) Physical and Chemical Handbook

negatively charged carboxylate group do not disperse ceramic particles in aqueous suspensions. Hydroxyl groups are also necessary. Citric acid was found to be an excellent dispersant by itself. Using it alone resulted in attaining high solids loading, concentrations greater than 50 vol.% of AKP-30 alumina particles and viscosities lower than 1,000 cPs.

From this study, it can be concluded that the hydroxyl group associated with the carboxylic acid increases the absorption of the acidic monomer molecules to the ceramic particle surface (adsorption measurements) and therefore increases the surface charge of the particles (zeta potential measurements up to -68 mV). This high surface charge results in a stronger charge repulsion between the particles and therefore provides better dispersion stabilization in the aqueous suspension.

(\* For a detailed information, see the preprint of a manuscript being prepared for submission for publication, given in the appendix.)

### iii. Future Studies

Current status of this portion of the investigation is that citric acid and other monomeric biogenic acids are currently in wide use in industry and medicine, therefore, they are inexpensive and can be readily produced by large scale microbial fermentation. They are non-toxic, dissolvable in aqueous solutions, and readily degradable.

- Our search for mechanisms of dispersion characteristics of monomeric biomolecules will continue during the next year.
- As discussed in this and other sections, their use in ceramic processing in conjunction with gelling agents will also continue in order to establish conditions for best results.
- The recognition of the importance of hydroxyl group in combination of carboxylate group(s) in enhancing dispersion demonstrated by this investigation may lead to the recognition and development of new biopolymers and monomers as effective, useful, and biodegradable dispersants for environmentally-sound materials processing; we plan to continue characterization of these macromolecules for feasibility of ceramic processing,
- In addition, we plan to demonstrate, during the next year, the feasibility of processing of dense-sintered ceramic systems using both synthetic surfactants and biomacromolecules and perform mechanical tests on the densified ceramics.
- Critical evaluation for environmental effects during the use of both the synthetic polymers and biomacromolecules for ceramic dispersion and packing will be demonstrated. These tests will include, toxicity, biodegradability, water solubility, burnability, and others.

In the following, we give a summary of the results of the work on  
**Biogenic Hydroxylated Carboxylate Monomers That Serve  
as Dispersants for Ceramic Particles.**

**i. Summary:**

A total of 20 biogenic carboxylic acids, which contain one to three negatively charged  $\text{-COO}^-$  group(s), were investigated as dispersion additives in the processing of ceramic particles. Only carboxylic acids one or more hydroxyl groups were able to disperse  $\alpha\text{-Al}_2\text{O}_3$  particles in aqueous suspensions. Detailed studies of the two carboxylic acids, citric acid and tricarboxylic acid were undertaken: measurements of zeta potential, particle packing, and dispersion adsorption characteristics indicated that carboxylic acids groups and hydroxyl groups are both important for dispersion of ceramic particles. Hydroxyl groups increased the adsorption of the molecules to the particles.

## **Introduction**

Currently, synthetic polymers from petrochemicals, such as polymethacrylic acid (PMAA) and polyacrylic acid (PAA) are used as dispersants (1). Similarly, previous work from this laboratory has shown that biologically produced polyelectrolytes such as kelp and bacterial alginate and bacterial polyglutamic acid also function as dispersants in ceramic processing (2, 3). However, synthetic and biological polymers have several major limitations: 1) polydispersant layers may be too thick to allow high packing densities, especially when the particle size is reduced to the nanometer size range; 2) the polyelectrolyte may not be compatible with useful gelling biopolymer matrices; and 3) commercial cost.

Furthermore, PMAA may contain formaldehyde, and PAA may contain residual amounts of acrylic acid. Moreover, PMAA and PAA are toxic and are produced from precursors that are toxic and/or carcinogenic.

This study was undertaken to investigate the use of biogenic monomers as dispersants. The compounds tested comprise a group of carboxylic acids and come from biological sources.

## **Results**

### **(1) Dispersion of alumina suspensions by carboxylic acids.**

A total of 20 carboxylic acids containing one, two, three or four carboxylate groups were evaluated for their dispersion behavior. Sedimentation volumes with AKP-30 alumina results are shown in Table 1. Tartaric, citric, and mucic acids produced the best dispersion with the

**Table I: Sedimentation volumes (cake height, mL) of selected carboxylic acids at various concentrations (dry weight basis [dwb] of the particle)**

	0	0.1%	0.3%	0.5%	1.0%	2.0%	3.0%	4.0%	6.0%
Glutamate				1.8		1.8			
Mannuronate				1.8		1.85			
Adipic acid			1.8	1.75		1.8			
Mucic acid				0.5		1.6			
Fumarate ( <i>trans</i> )				3.0		3.0			
Maleic acid ( <i>cis</i> )				2.5		2.5			
Succinate	1.85	2.4	2.45	2.7	2.7			2.4	
<i>meso</i> -Tartarate				0.55		0.6			
<i>d,l</i> -Tartarate	2.2	2.6	1.5	0.55	0.55			0.45	
Malate				0.7		0.7			
Malonic acid				2.0		2.05			
Tartronic acid				0.7		0.5			
Mesoxalic acid				0.8		0.5			
Glutarate				2.5		2.5			
Sebacic acid				2.0		3.5			
Tricarballic acid				1.7		1.2			
Aconitic acid ( <i>trans</i> )				1.8		1.75			
Nitrilotriacetic acid	2.25		1.9	1.45	1.1			0.9	
Citrate	2.0	2.0		0.5	0.5		0.4		0.4
Ethylenediamine tetracetate	1.8	1.75	2.0	2.0	2.0			1.9	

ceramic suspension and malic, tartronic, and mesoxalic acids provided some dispersion effect. From the molecular structure, it can be seen that tartaric, citric, mucic, malic, tartronic and mesoxalic acids all contain hydroxyl group(s). In contrast, succinic, maleic, tricarballic, aconitic, and adipic acids which are not satisfactory dispersants, do not contain this group. When the various organic acids that are structurally similar to one another are compared by molecular weight and pKa value, the hydroxyl group was the principal difference between those producing good versus poor dispersion.

Suspensions prepared with hydroxylated organic acids had the lowest viscosities. With citric or tartaric acids,  $\alpha$ -Al<sub>2</sub>O<sub>3</sub> (AKP-30 of Sumitomo Chemical, median size 0.4  $\mu$ m) particle suspensions having solid concentrations as high as 50 vol% could be prepared with a viscosity lower than 1 mPa.s. On the other hand, it was difficult to prepare ceramic suspensions with a solids concentration higher than 20 vol% when succinic, malic, tricarballic, aconic or adipic acids were used as dispersants (data not shown here).

## (2) pH effects and zeta potentials

Two carboxylic acids, citric acid versus tricarballic, were chosen for more detailed investigation of sedimentation properties and surface charge under various conditions. The sedimentation results of citric acid with various concentrations of the acids are shown in Figure 1. All samples (except the samples at pH 4) at pH 6, 8, 9, and 10 produced the lowest cake heights (about 0.5 ml in this test) when the concentration of citric acid in the suspension was increased to 0.3 % (dwb). At pH 4, the cake height was the lowest without any citric acid and eventually increased as the concentration of the acid increased to achieve a maximum at 0.4 %. After this, the cake heights of the samples plateaued at about 1.2 ml. As shown below, this low



packing is due to insufficient charge development on the particles with citric acid adsorption at this pH in spite of the fact that adsorption is strong.

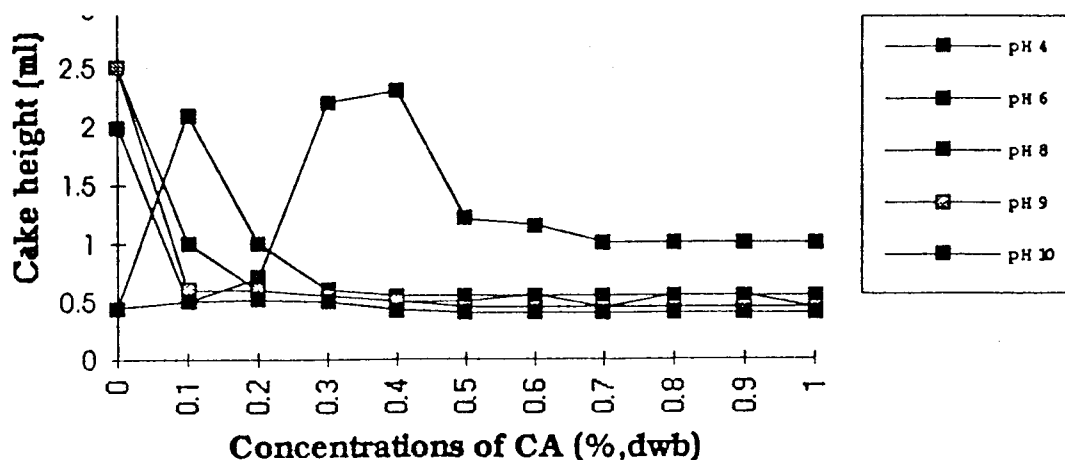


Figure 1: Cake heights of AKP-30 particle suspensions with citric acid (0.5%, dwb) under various pHs.

These dispersion behaviors correlate with and can be explained by zeta potential experiments. Figure 2 shows that with increasing citric acid concentration, the zeta potential decreases to zero (at pH 4 and 6) and then reverses sign and increases in the negative direction. In pH 8, 9, and 10 suspensions the zeta potential is near zero or already negative, so, as the concentration of the citric acid increases, the zeta potential continues to increase in the negative direction. Above 0.2% acid, the zeta potential approaches a nearly constant value of 65 mV (at pH 4 this is 33 mV). This value may reflect the state in which a saturated monolayer of citric acid occurs.

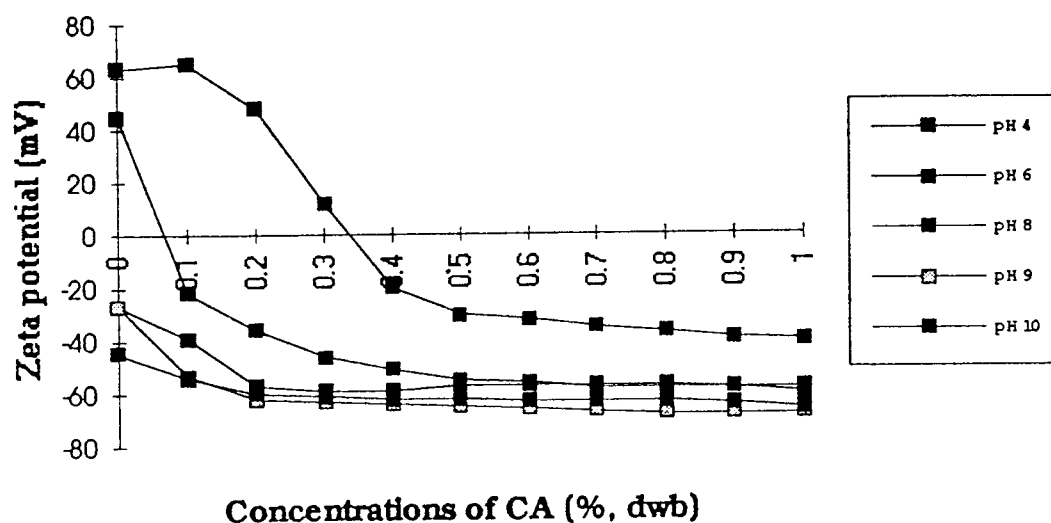
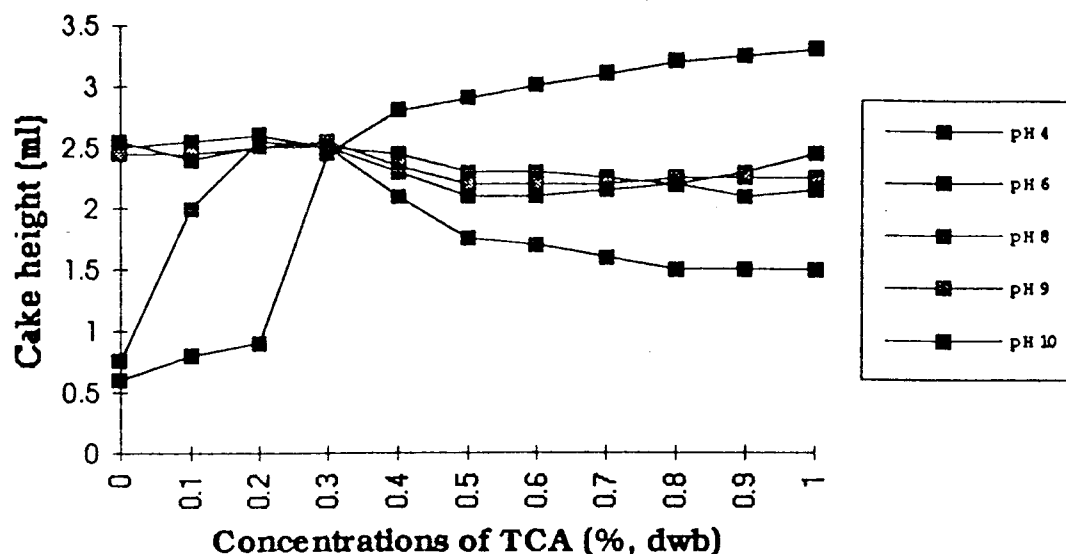
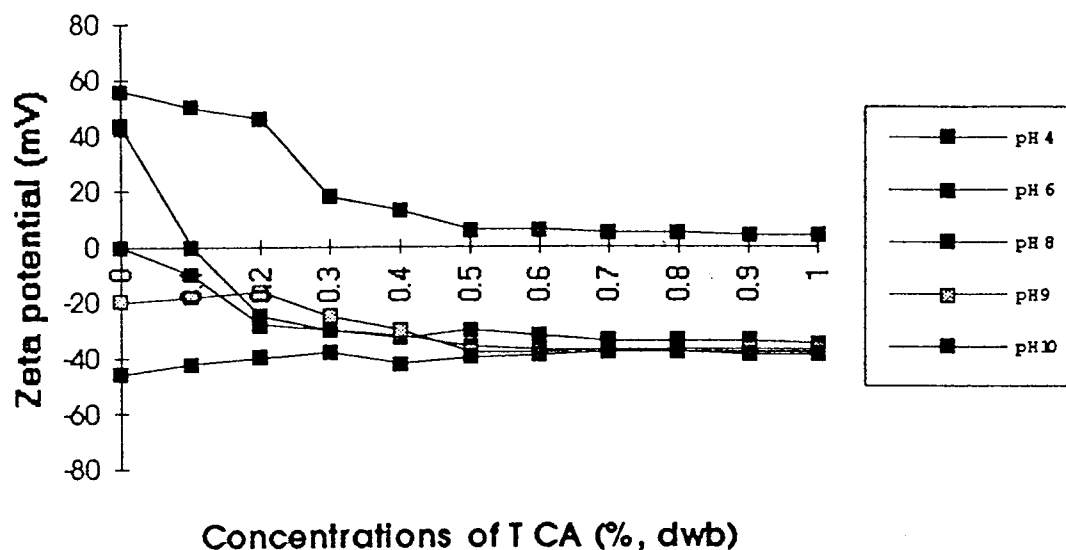


Figure 2: Surface charges of AKP-30 particles prepared with 0.5% (dwb) citric acid at different pHs.

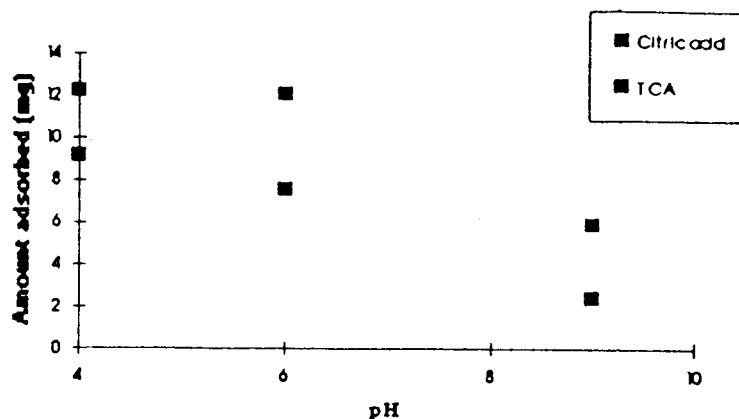


**Figure 3:** Sedimentation densities of AKP-30 particle suspensions when tricarballic acid was used as a dispersant with a concentration of 0.5% (dwb) versus pHs.



**Figure 4:** The surface charges of the AKP-30 particles prepared with tricarballic acid at a concentration of 0.5% (dwb) with different pHs.

Tricarballic acid did not produce the same packing densities as the citric acid (Figure 3) regardless of pH. At pH 4 the zeta potential changed from 55 mV to about 5 mV and never reversed sign, and the cake height increased from 0.6 mL to 3.5 mL (at 1.0%). At pH 6, the zeta potential decreased from about 43 mV to zero at about 0.2% concentration and then reversed sign and decreased to about -30 mV. This result is consistent with the sedimentation tests in which the cake height is first low (0.75 mL) and reaches its summit (2.5 mL) below 0.2% concentration and then decreases to around 1.5 mL but never decreases further although the concentration of the acid was increased to 6% (result not shown). At pH 8, 9, and 10, the zeta potential varies within the range of 0-40 mV, but the cake heights changed only slightly (from 2.7 mL to 2.3 mL).



**Figure 5:** Comparison of the adsorption of tricarballic acid and citric acid to 3.96 g AKP-30 particles

### (3) Adsorption studies

According to this study, it can be concluded that the higher particle surface charge results in a higher packing density and the hydroxyl group may produce an increase in the surface charge. How do the hydroxyl groups affect the particle surface charges?

One explanation could be that hydroxyl groups affect the adsorption of the acid to the particles. To verify this, tests evaluating adsorption of the acid to the particles were performed by titration. Figure 5 shows the adsorption of citric acid and tricarballic acid per 3.96 gram particles. The samples were examined at a concentration of 0.5% (dwb) at pH 4, 6 and 9. At each pH, the adsorption of citric acid was always greater than that of tricarballic acid. These results clearly show that the hydroxyl group associated with the carboxylic acid can somehow increase the adsorption of the acid to the particle leading to an increase in the particle surface charge (zeta potential), and therefore results in better dispersion. From Figure 5, it can also be seen that more acid was adsorbed at lower pH values than at higher pH. This agrees again with the results from the zeta potential and sedimentation tests. It can be explained as follows: at lower pH, the surface of the particle has more positive charges than at higher pH and therefore can attract more negatively charged carboxylic acid.

## Discussion

The results of this study show that all of the carboxylic acids tested cannot be used as dispersants. Only those containing hydroxyl group(s) disperse alumina particles in aqueous suspensions. Although some organic acids tested contain more than two carboxylic groups, they do not function well as ceramic dispersants without the presence of a hydroxyl group. This observation is in agreement with the recent results of Graule, et al (5).

Furthermore, it can also be stated that for compounds that contain the same number of charged groups and carbon atoms and have a similar molecular structure, the more hydroxyl groups the molecule has, the better it performs as a dispersant (e.g., tartronic acid and mesoxalic acid contain one hydroxyl group; tartaric, malic, maleic and succinic acids contain two hydroxyl groups; citric, tricarballic and aconitic acids contain three hydroxyl groups; mucic and adipic acids contain four hydroxyl groups). Compared with the effect of the hydroxyl group, the effect of a double bond between the carbon atoms is insignificant.

The explanation of how the hydroxyl group of the carboxylic acid affects the adsorption and the dispersion behavior is not clearly understood. One possibility is hydrogen bond formation

between substrate and adsorbate. A hydroxyl group on the carboxylic acid would increase the polarity of the molecule, thus increasing the adsorption by hydrogen bond formation or by other mechanisms. This increase of the adsorption is accompanied by an increase in the surface charge of the particles and therefore an increase in the dispersion capability.

It is also possible that a hydroxyl group associated with carboxylic acid could increase the affinity chelation of the acid to the particle surface and this could strengthen the reaction between the particle and dispersant. Or, a combination of these and other effects may play a role. Although citric acid is a chelating agent, it should be noted that other chelating agents, nitrilotriacetic acid and ethylenediamine tetracetate were not good dispersants (Table 1).

The chemical composition of biogenic carboxylic acids makes them suitable for diverse industrial processes. These monodispersants contain only carbon, hydrogen, and oxygen in their molecular structure and no phosphorous, sulfur, halides, or metals. Therefore, they would not leave an undesirable residue which could impart defects in sintered ceramic products. Additionally, because most of them are small monomers they readily dissolve in aqueous solutions, and unlike most polymers, they do not impart high viscosities to ceramic suspension when mixed with gelling agents for injection molding.

Citric acid and other biogenic acids are currently in wide use in industry and medicine. They are inexpensive and can be readily produced by large scale microbial fermentation (6). Moreover, they are non-toxic and biodegradable.

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## **2.3 The Use of Polymers in Ceramic Processing: A Comparison of Synthetic- and Bio-polymers for Safety Concerns**

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### **i. Objectives:**

In this part of the work, the objective was to initiate a study to investigate the types of specific damage that the synthetic polymers may cause to the environment when they are used as aids in the processing of ceramics; and they are compared with those of biopolymers. The following gives a summary of such an investigations. The detail of this investigation is given in the appendix which is the part of an MS thesis generated from this work. In the future, as part of the continuation of the present parent project, we plan to extend our studies to cover the use of biopolymers in-situ in the processing of ceramics, produce ceramic parts and test their mechanical properties as compared to those produced using the synthetic polymers (as part of Ms. Hashemifar's Ph.D. Project). The proposed areas are listed at the end of this section.

### **ii. Introduction:**

The fundamental requirement for preparation of high density ceramic compact is that submicron-sized particles be completely dispersed in the solvent system and exhibit no agglomeration. Furthermore, they should retain the state of high dispersion throughout the consolidation state. In this capacity, polyelectrolytes acts as dispersants or defloculants in colloidal processing of ceramics.

There are two major classes of these additives: synthetic polymers, for example PMMA, and biopolymers, such as alginates. The synthetic polymers, i.e., polymetacrylic acid, polyelectrolyte has been used in steric and/or electrosteric applications. A comparison of the behavior of PMMA and alginate when used as a suspension-enhancing aids through electrosteric stabilization, in the polyelectrolyte/alumina system is presented. It is shown that alginate as a dispersant has comparable properties to the PMAA and its rheological behavior, sensitivity to pH variation, polymer molecule weight, concentration, and viscosity are comparable polyelectrolytes.

Comprehensive survey of data on health hazards (skin and respiratory irritation and carcinogenicity), storage and handling (corrosivity, toxicity, and flammability), chemical reaction (self-reaction, water, and other chemicals), and toxicity of decomposition products, shows that the use of alginate, a naturally occurring biopolymer instead of PMMA, leads to a safer environment. This is based on the assessment of Degree of Environmental Damage (DED) factor of the polymers used as dispersants in ceramic processing. While retaining comparable processing properties, alginate has a DED factor of 1030 compared to 1812 for PMMA, and, hence, provides an environmentally-benign processing.

### **iii. Summary of the Environmental Issues Associated with PMMA and Alginate:**

#### **Comparative Analysis:**

This section summarizes the environmental issues for PMMA and alginate; the production and polymerization of PMMA and alginates are discussed in the extended analysis presented in the appendix. In order to take into consideration the environmental aspects of all the materials involved in the processing two different biopolymer and synthetic polymer (Na-alginate and PMMA), we need to conduct a comparative analysis between their various characteristics. The different aspects investigated are as follows (see details in the appendix):

- a. Health hazards
- b. Storage and handling
- c. Chemical reactivity
- d. Toxicity of decomposition products
- e. Waste disposal

Table - I shows environmental impact ratings for the 30 different materials which are involved in the processing of alginate, MAA, and its polymer PMMA. Materials used in the processing of alginate and PMMA are both excluded in this rating (this will be study for future). The different routes of production were separated from each other with two horizontal lines. The rating information for each of the categories was taken from materials safety data sheets of various companies as well as other sources. The lower the rating number, the less harmful and more environmentally friendly the material is. Composition products are also given along with their toxicity ratings. The table gives all the environmental data discussed.

Table I A Comparison Between PMAA and Alginate and Their Related Materials for Safety Concerns

No.	Material	Health Hazards			Storage and Handling			Chemical Reactivity			Toxicity of Decomposition Products	Waste disposal <sup>h</sup> methods <sup>2</sup>	Refs.
		Skin	Respiratory	Carcinogenicity	Flammability	Toxicity	Corrosivity	Other Chemicals	H <sub>2</sub> O	Self-reactor			
1	Polymethacrylic acid PMAA Sodium salt	1	1	0	0	1	0	n/a	n/a	n/a	3 CO, CO <sub>2</sub> , Sodium Oxide	14	1, 2, 3, 4
2	Methacrylic acid (monomer)	3	3	1 <sup>a</sup>	2	2	1	2	2	3	3 CO, CO <sub>2</sub>	3	2, 5, 6, 7, 8, 9
3	Acetone	2	2	1 <sup>a</sup>	3	2	0	2	0	2	3 CO, CO <sub>2</sub>	1	2, 7, 8, 10, 11, 12
4	Hydrogen Cyanide	3	4	3	2	4	3	3	0	3	3 CO, Nitrogen oxide, Hydrogen cyanide	29	7, 11, 13
5	Acetone Cyanohydrin	3	3	1 <sup>a</sup>	1	3	2	3	2	3	3 CO, Nitrogen oxide, Hydrogen Cyanide	3	2, 11, 14
6	Carbon monoxide	2	2	1 <sup>a</sup>	2	3	0	0	0	0	3 CO, Nitrogen oxide, Hydrogen Cyanide	28	2, 11, 15
7	Methanol	3	3	2 <sup>b</sup>	4	3	0	1	0	0	4 CO, Formaldehyde	3	2, 11, 16, 17

Table I (continued)

No.	Material	Health Hazards			Storage and Handling			Chemical Reactivity			Toxicity of Decomposition Products	Waste disposal methods <sup>h</sup>	Refs.
		Skin	Respiratory	Carcinogenicity	Flammability	Toxicity	Corrosivity	Other Chemicals	H <sub>2</sub> O	Self-reactor			
8	Methyl Methacrylate	2	2	2 <sup>b</sup>	1	1	1	2	0	3	CO <sub>2</sub> , CO <sub>2</sub>	1	2, 11, 18, 19
9	Ammonia	3	3	1	1	3	3	2	3	2	Ammonia	28	2, 11, 15, 20, 21
10	Tert-butylalcohol	2	2	0	2	3	0	2	2	0	CO <sub>2</sub> , CO <sub>2</sub>	14	11, 22
11	Isobutylene	2	2	0	2	3	0	0	0	0	CO <sub>2</sub> , CO <sub>2</sub>	30	23
12	Methacrolein	3	3	1 <sup>a</sup>	2	3	3	2	3	1	CO <sub>2</sub> , CO <sub>2</sub>	1	2, 24
13	Propylene	1	1	2 <sup>b</sup>	2	3	0	1	0	1	CO <sub>2</sub> , CO <sub>2</sub>	28	11, 18, 19, 25
14	Isobutyric acid	3	3	2	2	2	2	2	0	0	CO <sub>2</sub> , CO <sub>2</sub>	3	1, 2, 26
15	Hydrogen fluoride	4	4	3 <sup>c</sup>	0	4	4	2	2	0	Hydrogen Fluoride <sup>4c</sup>	6	2, 11, 27
16	Boron trifluoride	3	3	1 <sup>a</sup>	1	3	4	4	4	0	Hydrogen Fluoride <sup>4</sup>	28	1, 2, 15
17	Propionic acid	3	3	2 <sup>b</sup>	1	2	1	2	0	0	CO <sub>2</sub> , CO <sub>2</sub>	3	2, 11, 18, 29



Table I (continued)

No.	Material	Health Hazards			Storage and Handling			Chemical Reactivity			Toxicity of Decomposition Products	Waste disposal <sup>b</sup> methods <sup>2</sup>	Refs.
		Skin	Respi-ratory	Carcino-genicity	Flamm-ability	Toxi-city	Corro-sivity	Other Chemicals	H <sub>2</sub> O	Self-reactor			
18	Ethylene	3	3	2 <sup>b</sup>	2	1	0	1	0	2	CO, CO <sub>2</sub>	28	2, 11, 18, 19, 29
19	Propionaldehyde	3	3	1 <sup>a</sup>	2	2	1	2	0	1	CO, CO <sub>2</sub>	1	1, 30
20	Ammonium Peroxy Disulfate	3	3	1 <sup>a</sup>	0	2	1	2	0	0	Nitrogen Oxide, Sulfur oxide	10	11, 31
21	Toluene	2	2	2 <sup>b</sup>	2	2 <sup>c</sup>	0	0	0	0	Nitrogen Oxide, Sulfur oxide	1	2, 8, 10, 11, 32
22	Hexane	2	2	2 <sup>b</sup>	3	2 <sup>f</sup>	0	0	0	0	Nitrogen Oxide, Sulfur oxide	1	2, 7, 10, 11, 33
23	Alginate acid sodium salt	1	1	1 <sup>a</sup>	0	0	1	1	0	0	CO, CO <sub>2</sub> , Nitrogen oxide	2	2, 9, 34
24	Hydrochloric acid	3	3	1 <sup>a</sup>	0 <sup>d</sup>	3	4	2	0	0	Hydrogen chloride gas	4	2, 8, 11
25	Sodium Carbonate	0	1	0	0	0	0	1	0	0	Hydrogen chloride gas	4	2, 8, 35
26	Kieselguhr Diatomaceous earth	1	1	1 <sup>a</sup>	0	0	0	1	0	0	Silicon oxide	14	8, 36
27	Perlite	1	2	1 <sup>a</sup>	0	0	n/a	1	0	0	n/a	15	7, 37

Table I (continued)

No.	Material	Health Hazards			Storage and Handling			Chemical Reactivity			Toxicity of Decomposition Products	Waste disposal methods <sup>h</sup>	Refs.
		Skin	Respiratory	Carcinogenicity	Flammability	Toxicity	Corrosivity	Other Chemicals	H <sub>2</sub> O	Self-reactor			
28	Polyacrylamide	1	1	1 <sup>a</sup>	0 <sup>g</sup>	3	0	1	0	0	3 CO, CO <sub>2</sub> , Nitrogen oxide	2	2, 8, 38
29	Sodium hypochloride	3	3	1 <sup>a</sup>	0	3	2	2	0	0	4 Hydrogen chloride gas	10	2, 11, 39
30	Calcium chloride	2	1	1 <sup>a</sup>	0	0	0	1	0	0	4 Hydrogen chloride gas	4	2, 8, 11, 40

a) Either not listed as carcinogen by NTP (National Toxicology Program) and OSHA (Occupational Safety and Health Administration), or has not been evaluated by IARC (International Agency for Research on Cancer).

b) Check Ref. #19, Page 16-38.

c) Possible mutagen.

d) Flammable gas may be produced on contact with metals.

e) Several cases of sudden death are to be noted, especially in the case of children or adolescents given to "glue sniffing" or inhaling fumes from adhesives containing toluene among other solvents, resulting from cardiac arrest due to ventricular fibrillation with loss of catecholamines. Persons exposed to toluene should receive a periodic medical examination at intervals of 6-12 months; the clinical examination should include haemocytometric testing and a thrombocyte count in view of the possibility that toluene may contain a certain proportion of benzene. If ventricular fibrillation occurs, catecholamines must be administered<sup>8</sup>.

f) Suspected neurotoxin.

g) Under fire conditions material may decompose to form flammable and/or explosive mixture in air.

h) See Appendix A.

### The Degree of Environmental Damage "DED:

In order to facilitate the comparison of the health hazards, storage and handling procedures, chemical reactivity and toxicity of the decomposition products of all the different materials given in the previous table, a factor termed as degree of environmental damage was defined to measure semi-quantitatively the damage each of these materials has on the environment. Table -II shows different DOD values for every single category of environmental aspect of materials related to both alginate and PMMA.

In calculating the DED's, the information given in Table -I were used. For example, in the case of skin irritation for alginate, 12.5% of materials were rated 0 and 50%, 12.5%, 25%, and 0% were consecutively rated 1, 2, 3, and 4. DED equals to the summation of the percentages times their related ratings. In this case, for example:

$$\text{DED} = (12.5 \times 0) + (50 \times 1) + (12.5 \times 2) + (25 \times 3) + (0 \times 4) = 150.$$

As shown in the table, DED for all PMMA-related categories is higher than the one for alginate. Therefore, the DED factor, based on the data presented in the table, gives the degree of damage of production and use of PMMA, a synthetic polymer, as a processing aid in ceramic processing, compared to that of alginate, a biopolymer.

#### iv. Future Research:

This will include the following:

- The dispersion behavior and environmental aspects of bacterial alginate and other biopolymers should be compared to PMMA;
- Other synthetic polymers such as PAA should be compared with naturally occurring biopolymers
- Production and utilization costs of PMMA and alginate should be compared
- Energy consumption in production and utilization of biopolymers and synthetic polymers should be compared
- Water pollution caused by the polymers and other related materials involved in their production should be investigated
- biodegradability of the polymers and their related compounds should be investigated
- recycling factors should be taken into account in environmental impact
- *These factors (and perhaps others) incorporated into the DED factor described here should be used to quantitatively assess the safety degree of processing aids in the synthesis of all engineering materials.*

Table II Overall Degree of Environmental Damage "DED" of PMAA and Alginate Group of Materials

Characteristics		Rating Percentages*		Alginate (Sodium salt) %					DED***	PMAA (sodium salt) %					DED***
				Alginate (Sodium salt) %						PMAA (sodium salt) %					
				0	1	2	3	4		0	1	2	3	4	
Health Hazards	Skin	12.5	50	12.5	25	0	150	0	9	32	54.5	4.5	255		
	Respiratory	0	62.5	12.5	25	0	163	0	9	32	50	9	259		
	Carcinogenicity	12.5	77.5	0	0	0	78	13.6	41	36.4	9	0	141		
Storage and Handling	Corrosivity**	50	12.5	12.5	0	12.5	88	45.5	22.7	9	13.6	9	177		
	Toxicity	62.5	00	0	37.5	0	113	0	13.6	36.4	41	9	246		
	Flammability	100	0	0	0	0	0	13.6	22.7	54.5	4.5	4.5	163		
Chemical* Reactivity	Self-reaction	100	0	0	0	0	0	50	18.2	9	18.2	0	41		
	Water	100	0	0	0	0	0	63.6	0	18.2	9	4.5	81		
	Other chemicals	0	75	0	25	0	150	18.2	13.6	50	9	4.5	159		
Toxicity of Decomposition Products**		0	0	12.5	37.5	37.5	288	0	0	0	81.8	13.6	300		
TOTAL "DED"							1,030	TOTAL "DED"					1,812		

\* All the percentages have been rounded to the nearest 0.1.

\*\* If the summation of the percentages is less than 100, it means that one data for one of the materials has not been available.

\*\*\* DED (Degree of Environmental Damage) for each category is The summation of multiplication of factors and rating percentages.

## 2.4 Mechanical Properties of Biological Composites

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(<sup>+</sup> was a post-doc; now left; \*now at Chemical Engineering, Princeton University)

### i. Summary

Nature produces ceramic-polymer laminates with hierarchical structures at nanometer scale. These materials exhibit exceptional strength and toughness compared to the monolithic components. In this investigation, the deformation mechanisms which contribute to the strength and toughness of biolaminates were studied. Examination of Vickers indentations (2 to 600 N) and associated cracking in a nacre sample sectioned perpendicular to layers revealed large anisotropic damage zones and periodic deformation bands. Two types of banding were observed: periodic large bands that were separated by numerous smaller ones. Our studies indicate that the damage zones and deformation bands were controlled by the microarchitecture of the laminate, which is further confirmed by the surface topography, studied by atomic force microscopy, that displayed similar periodicity's. The large plastic deformation zone, in addition to the ligament formation and tortuous crack path contribute significantly to the mechanical properties of the laminate.

### ii. Justification and Objectives

One of the important aspects of biomimetics is to obtain lessons from the study of the biological composites and assess the influence of the organism through the details of structural design and behavior of the biocomposite under various engineering characterization tests. To achieve this objective in this project we have initiated a study which involves a critical and detailed comparison of biogenic and geological calcium carbonates. From the differences of test results and the evaluation of the fracture behavior, we aim to extract the influence of the organism over the biogenic composite compared to the geological samples, the case where no organic matter is present. In order to achieve design criteria for future biomimetic synthetic composites in terms of their structures for improved mechanical properties, we are investigating the structure of both the nacre and prismatic sections of mollusk shells of graded structures (i.e., gastropods, bivalves and cephalopods) and correlating them with their superior mechanical properties over current advanced ceramics and composite systems. Our long-term objective in this study is to assess the

#### iv. Results

### Comparison of Mechanical Properties of Biogenic and Synthetic (geological) Calcium Carbonates and Theoretical Understanding of the Mechanical Property Increases in Biocomposites:

During the latter part of this period of the project ( Summer 1994), we have started to evaluate the microhardness properties, including the crack propagation behavior, for a better insight into the influence of the higher order in the microstructural makeup and the presence of organic in the biogenic calcium carbonates as compared to geological single crystals. With this study, we have now, for the first time in the literature, take the whole shell as a single system, rather than concentrating only on its components (prismatic and nacreous sections separately). The objective is to achieve an integrated micromechanical picture of the shell-system with the aim of using the learned lessons as models for future engineering systems for impact resistant applications.

The first in the series of experiments we plan to conduct has been the microhardness tests (the a more complete list of future investigation is given at the end of this section). Abalone was taken as the first system for study. In this case, there are four sets of microhardness measurements of interest. Each of the two microstructures, i.e., nacre and prismatic sections, present in the sample has at least two theoretically distinct directions in which properties will deviate; edge-on and face-on directions. Face-on is correlated with the top and bottom surfaces , i.e., [001] direction in the aragonite (orthorhombic) lattice structure. Edge-on is correlated with either of { 100}, [010] and [110] direction of the lattice. In the prismatic regions, the crystal lattice directions (i.e., the calcite, rhombohedral) are less defined on face-on and edge-on; therefore, these terms are meant, for now, to describe directions parallel to those in the nacre section (we are in the process of establishing these directions in the prismatic section using microdiffraction studies in the transmission electron microscope). Therefore, the abalone sample yields four primary sets of data: nacreous: edge-on and face-on; and prismatic, edge-on and face-on. In order to compensate for the deviance found in the preliminary tests the load used has been normalized for all indentations as has the loading duration.

In all cases, 50 grams of load was applied to each direction for 15 seconds, and the following average values (at least of eight readings):

Nacre:	edge-on:	173.6
	face-on:	170.0
Prismatic:	edge-on:	214.6
	face-on:	202.6

From the data, it is clear that the two sections exhibit distinctive differences (about 25%) in the microhardness values. This difference is what would be expected of an impact resistant structure; i.e., thin and hard section on the outside (prismatic) and the thick and softer (but tougher) section on the inside (nacre). (The important microhardness information combined with the size of each sections will be incorporated in our future theoretical micromechanics model).

What cannot be distinguished from this data is the difference in the microhardness by direction within a given material. This is especially puzzling in the nacre section since atomic arrangement in two directions in the orthorhombic lattice are quite different. The influence of the microarchitectural organization of the aragonite platelets and the presence of thin organic macromolecular film surrounding them will have to be taken into consideration in the future studies. The comparison of these results with those from geological single crystal aragonite samples is underway to assess the influence of organism over the properties of the structure.

On the other hand, the difference of 5% in the microhardness values of the prismatic section in two orientations is not significant and is somewhat expected. It is believed (but not yet fully established yet) that the directional organization of the calcite crystals is crystallographically homogeneous (i.e., directional properties of calcite along [001] and one of the [110] directions may not be significantly different). Nevertheless, we plan to evaluate these directional properties of calcite using the large single geological crystals in the near future.

## High Resolution Imaging Studies of Fracture Behavior of Nacre:

The majority of mechanical property data to date has been collected from "dry" abalone shells (purchased from shell dealers and out of sea water for an extended period of time 1-2 years). The results from flexural strength and indentation tests show a strong influence of strain rate on the mechanical behavior of nacre (Figs. 1 and 2). The flexural strength of nacre increases with increasing strain rate, due to the strain rate dependence of biopolymers. This effect may be amplified due to the constraint of the surrounding ceramic platelets. The indentation behavior of Nacre as characterized by acoustic emission shows a similar effect of strain rate. The number of acoustic events recorded during indentation increases with increasing indenter speed. At slow strain rates there is sufficient time for "quiet" deformation (perhaps platelet sliding) to accommodate significant plastic strain introduced by indentation until a load of 17 Kg is reached. At fast strain rates, acoustic events are recorded at low load levels (2.5 kg) as the composite is unable to quietly accommodate strain occurring at high rates.



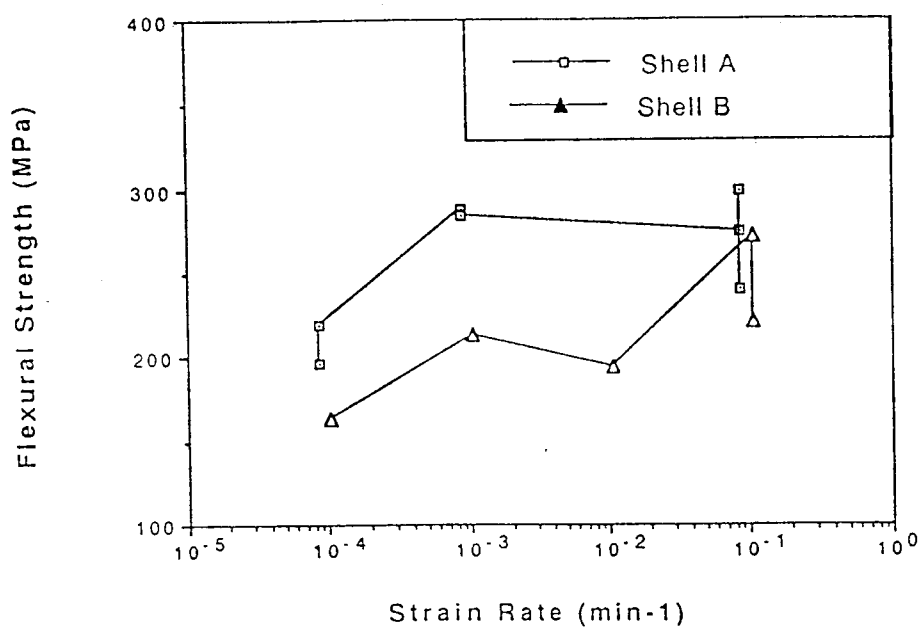


Figure 1 - Flexural strength versus strain rate of nacre of abalone.

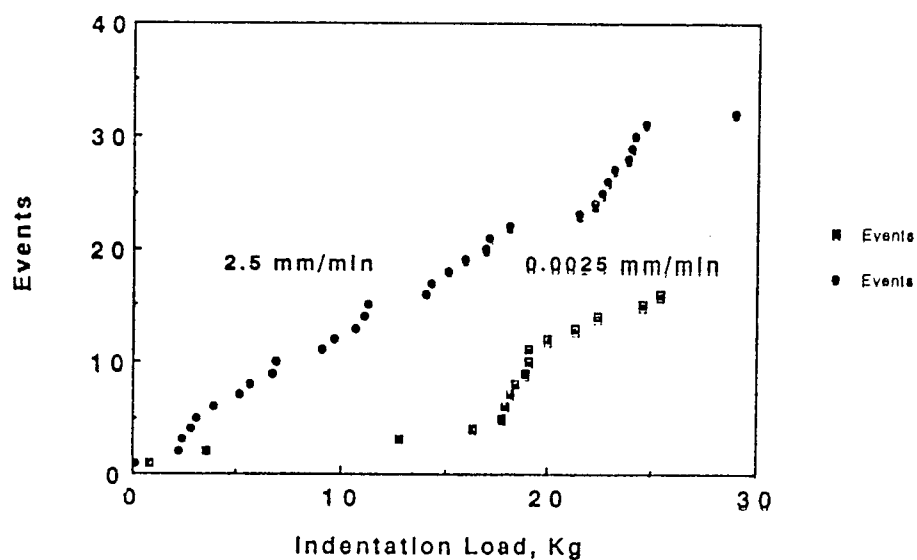


Figure 2 - Effect of strain rate on the acoustic emission during the fracture of nacre.

The damage zone surrounding Vicker's indentations in the edge orientation of nacre has been characterized by several methods including scanning electron microscopy, atomic force microscopy and light optical methods. The damage occurs in an anisotropic manner as dictated by the structure of nacre. Damage extends much further in the direction perpendicular to the aragonite platelets (Fig. 3). The damage zone is manifested as an hierarchical arrangement of steps on the surface extending out from the indentation (Fig. 4). The large surface steps as seen in optical interference microscopy (Fig. 3) consist of several smaller steps due to single platelets sliding. A comparison of the size of damage zone resulting from indentation between nacre and its major (95%) constituent aragonite (geological single crystal in same orientation as single crystal platelets in Nacre) show that nacre can absorb significantly greater damage without cracking than pure aragonite (Fig. 5).



Figure 3 - Damage zone in the vicinity of an indentation made in the edge-on configuration in nacre.

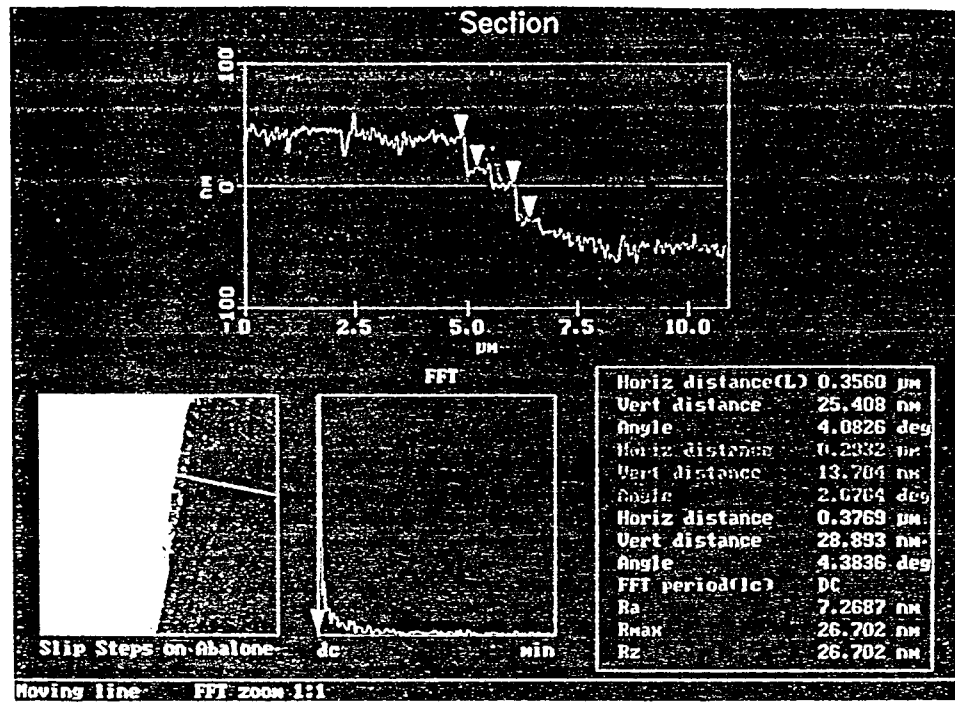


Figure 4 - Atomic force microscopy studies of the steps that are created in the damage zone next to an indentation in nacre tested edge-on. Note that the step size (arrows) is the same as the thickness of the aragonite platelets implying that sliding is taking place forming surface striations.

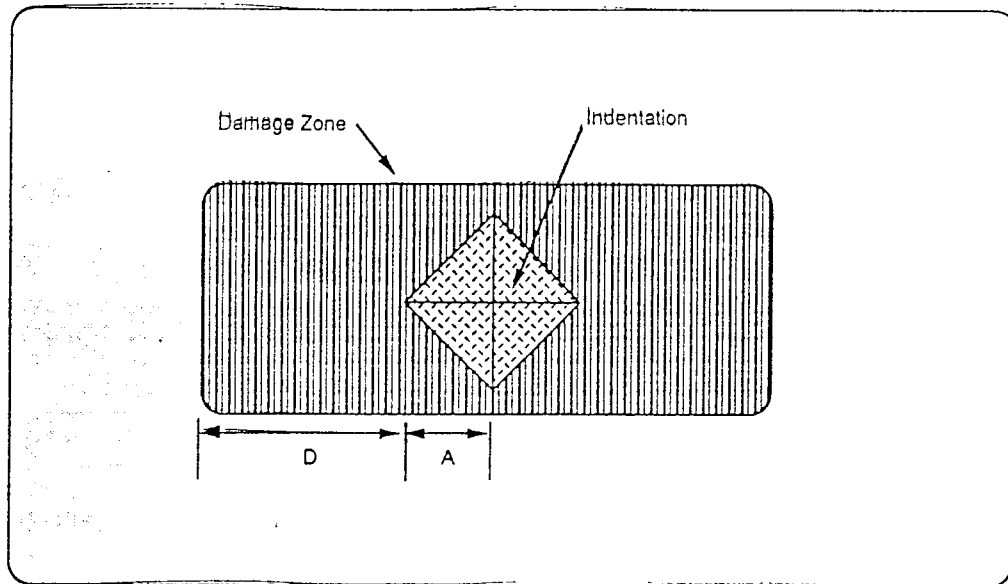


Figure 5 - Schematics and comparison of damage zones in geological aragonite and biogenic aragonite (nacre).

Fresh abalone shells collected from the coast of northern California and kept in sea water at 5°C are currently being machined into mechanical test specimens. Characterization of the drying behavior of the wet nacre is nearing completion. Thermogravimetric analysis (TGA) of fresh wet nacre and dry nacre indicate significant differences in water content. The kinetics of water loss during drying is being analyzed by monitoring weight loss as a function of time, temperature and specimen size and orientation.

#### **v. Future Directions:**

There are several parallel directions to take:

- o- Influence of the organic matrix on the deformation and failure mechanisms of nacre (dry vs. wet samples, strain rate sensitivity, damage zone size)
- o- Structure and properties of inorganic/organic interfaces (e.g., peel test)
- o- To investigate the failure mechanisms, acoustic emission properties of nacre during fracture and failure (in collaboration with Dr. T. Dickinson, Washington, State University) will be determined to probe crack initiation and propagation within matrix, at interfaces, or within the inorganic phase, at different stress levels (organic or inorganic sources of failure).

## 2.5 Fine Crystalline Particle Formation in a Microbiological System: Formation of Magnetite in *Aquaspirillum magnetotacticum*.

Nancy B. Pellerin, Tao Ren, James T. Staley, and Mehmet Sarikaya, Departments of Microbiology and Materials Science and Engineering, University of Washington

### i. Justification and Objectives

Some bacterial species produce magnetite ( $\text{Fe}_3\text{O}_4$ ) or pyrite ( $\text{Fe}_3\text{S}_4$ ) particles that are organized in the form of strings along their cell, and act as their compass. The particles are 300 to 600 Å in diameter and, therefore, in the superparamagnetic regime. Each bacterial species have particles with unique shape, but all the particles are perfect crystals without lattice defects, such as dislocations or planar internal interfaces. It is known that particles form in "sacks" called magnetosome membranes which is thought to control the particle formation. The aim in the present research is to decipher the mechanism of particle formation in biological closed membranes (reaction vessels), and from the learned principles, attempt to produce synthetic ultrafine magnetic (and other) particles either using proteins or other macromolecules. Our long-term goal is to learn biomimetics ways of producing magnetic, semiconducting, and optical particles for technological application.

Particles synthesized by traditional and advanced techniques currently available to us materials scientists (such as surfactant or vesicle mediated particle formation, or coprecipitation) lack the qualities of bacterial particles in that they are loosely crystalline containing lattice defects, inhomogeneous elemental concentrations, irregular shapes, and sizes. It is desirable, therefore, to learn from biology the way the organisms can form particles whose unique structure and properties seemed to be controlled by the organism.

Our short term objectives involve the investigation by studying the structural development of the particles in bacteria by microscopy and microbiological means in an attempt to learn the mechanisms of particle formation within magnetosome membranes in *A. magnetotacticum*.\*. The long term objectives include the use components from the system (purified membranes and proteins) to produce thin film or vesicle mediated particles, and understand the system well enough to tailor it to the production of particles of specific size/shape (morphology) and composition (genetic analysis).

- o- Thin sections were prepared after embedding in plastic resin. The sections were stained with lead citrate and uranyl acetate before examination as above.

### (iii) Future Work

#### A. Ion Transport

- How is the iron transported across the membrane into the reaction vessel?
- What is the iron transport protein and where is it located?

Magnetosome membrane proteins will be purified on SDS acrylamide gels. The iron binding protein(s) will be identified by their capacity to bind iron. The purified protein will be sequenced and from the cDNA a fragment will be used to produce antibody. These antibodies can be used:

- to label (using immunogold techniques) the proteins in intact membranes for TEM visualization. These same techniques can be used to label and visualize other membrane proteins.
- to collect and purify magnetosome membranes not yet containing particles. These membranes can be used for studies of iron transport, that is, what conditions and other components are necessary for magnetite production? These membranes may also be used as substrates for thin film and/or vesicle mediated production of particles.

#### B. Genetic Control

Some of the difficult questions are

- What are the genes controlling magnetite formation?
- Can they be manipulated so as to produce other products?

The fundamental understanding of any biological process requires that the genetic components of the system be identified, as well as the structural and functional components. In order to achieve this long term goal we have begun by isolating mutants defective in magnetite production. Such mutants are useful in identifying gene products involved in the process of mineralization. To date, we have isolated one such mutant and are continuing efforts to isolate others.

## 2.6 Lessons From Biology: Structures and Properties of Biocomposites: Crystalline Structure, Morphology, and Shape In Mollusks - Structural Studies of Nacre in Abalone as a Model for Biomimetic Research

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\* Now at Battelle Pacific Northwest Laboratories, Richland, WA.

### i. Summary: Hierarchically Structured Natural Ceramic-Polymer Composites

Biological hard tissues, such as bone, dentin, and seashells, are composite materials incorporating both the inorganics (phosphates and carbonates) and organics (macromolecular structural units including proteins).<sup>1-5</sup> These materials have unprecedented physical properties, which are often multifunctional due to their highly ordered structures through the dimensional scale from molecular to submeter.<sup>5</sup> Biological composites are a source of inspiration<sup>6</sup> for design and processing of synthetic materials based on both their structure, *biomimicking*, and processing, *bioduplication*.<sup>7</sup> In this presentation, we will discuss our current work that focuses on nacre section of the abalone and pinctada (oyster) shells where the hard tissue has a *brick and mortar* structure at the submicron and nanometer scales, respectively. Here the bricks are aragonite (orthorhombic  $\text{CaCO}_3$ ) constituting over 95 v/o, and mortar as a composite of macromolecules and proteins. The material has a combination of high strength (180 MPa/(g/cc)) and fracture toughness (8 MPa-m<sup>1/2</sup>) which are due to the characteristics of interfaces, and structures and properties of both the inorganic and the organic components.<sup>4,5</sup> Studies on the morphology and crystallography, by high resolution TEM, revealed that platelets are multiply tiled based on hierarchical twinning.<sup>5</sup> This suggest that the active sites for nucleation in the organic matrix has a pseudo-hexagonal ultrastructure and that the template growth mechanism is operational during nacre formation. Furthermore, based on growth pattern on a wide dimensional scale, the structure of the organic may have a direct consequence on the final shape formation of the shells.<sup>8</sup> We will, furthermore, discuss the current understanding of the biochemistry and structures of the organic matrices based on the results in the literature and the collaborative groups.

### C. Mechanism of Particle Formation

What are the sequential stages in nucleation and growth of particles within the membranes?

The composition and structure of the newly nucleated particle, and its subsequent growth, and in parallel, the changes in the membrane (its size, shape and composition) are central factors in the elucidation of the mechanisms of particle formation in membrane bound systems. A long term objective is to use this knowledge for the formation of nanoparticles in synthetic vesicles and micelles.

Initially we planned to study magnetite formation in *Aquaspirillum magnetotacticum*, an organism that we are growing in the laboratory. However, this bacterium is very difficult to cultivate and study. Therefore, in parallel to this existing investigation, our efforts are now also directed toward the isolation of new magnetic strains.

The objectives of this parallel work are as follows:

- Isolation and description of new magnetic bacteria,
- Electron microscopy of the structure of the magnetite particles,
- Isolation, purification, and characterization of magnetosome membrane,
- Identification of magnetite nucleation material.



## ii. Objectives and Justification

We plan to investigate the structure, crystallography, and morphology of nacre structure of mollusk shells, in particular, that of abalone, in order to obtain structural criteria for the design of novel materials based on biomimetic approach. Nacre is a ceramic-polymer composite found in mollusk shells; it has a uniquely ordered microstructure and unusual mechanical properties, such as high fracture toughness and strength.<sup>2,4,5</sup> Mollusk shells adapt elegant shapes depending upon the species and the environment they are grown in, although different shells are made of the same basic mathematical structure.<sup>5</sup> Investigation of this relatively simple biomaterial is performed with the aim of future possibilities of physical property control through tailoring of microstructure, in-situ fabrication, low temperature processing, and net shape forming novel technological materials. The first year's objectives in this part of the research have been to:

- investigate the development of the crystalline structures and morphological features of the inorganic phase units, i.e., aragonite,  $\text{CaCO}_3$ , platelets;
- Study the formation of the morphology of the inorganic crystals;
- The relationship between the crystalline structures of the organics and the inorganics (mechanism of biomineralization),
- investigate the relationship between the crystalline structure, morphology of the crystals, and the overall shape formation of the shells, i.e., morphogenesis (lessons from biology on the net shape forming of technological materials),

We focused our efforts on the first two of the items above; we initiated research on the third item, i.e., shape formation of the shells, during this period of the research.

## iii. Approach

The general approach of this work is to study the crystalline structures of nacre by electron microscopy from nanometer to millimeter, on a continuous scale, by imaging (by electron microscopy and scanning probe microscopy techniques) and electron diffraction. TEM samples are prepared by low temperature ion beam milling to preserve both short and long range order of the inorganic phase. Samples are coated with thin carbon films and studied with a low temperature sample holder (with the sample temperature being  $-170^\circ\text{C}$ ) to minimize damage of the structure due to the electron beam bombardment.

#### iv. Results

##### iv.1. Morphology and Crystallography:

Previous studies revealed that the platelets are generally aligned along the c-axis of the orthorhombic unit cell of aragonite, but it has been suggested that the platelets are grown randomly in the a-b plane.<sup>9</sup> This study, however, indicated that the platelets in the a-b plane are actually related to one another by a {110} twin relationship.<sup>5</sup> Either 90° and 60° twin boundaries have been observed in this study. Fig. 1a-b show a six fold arrangement of the twin platelets and Fig. 1c shows a diffraction pattern with two superimposed diffraction patterns taken on the twin boundary.

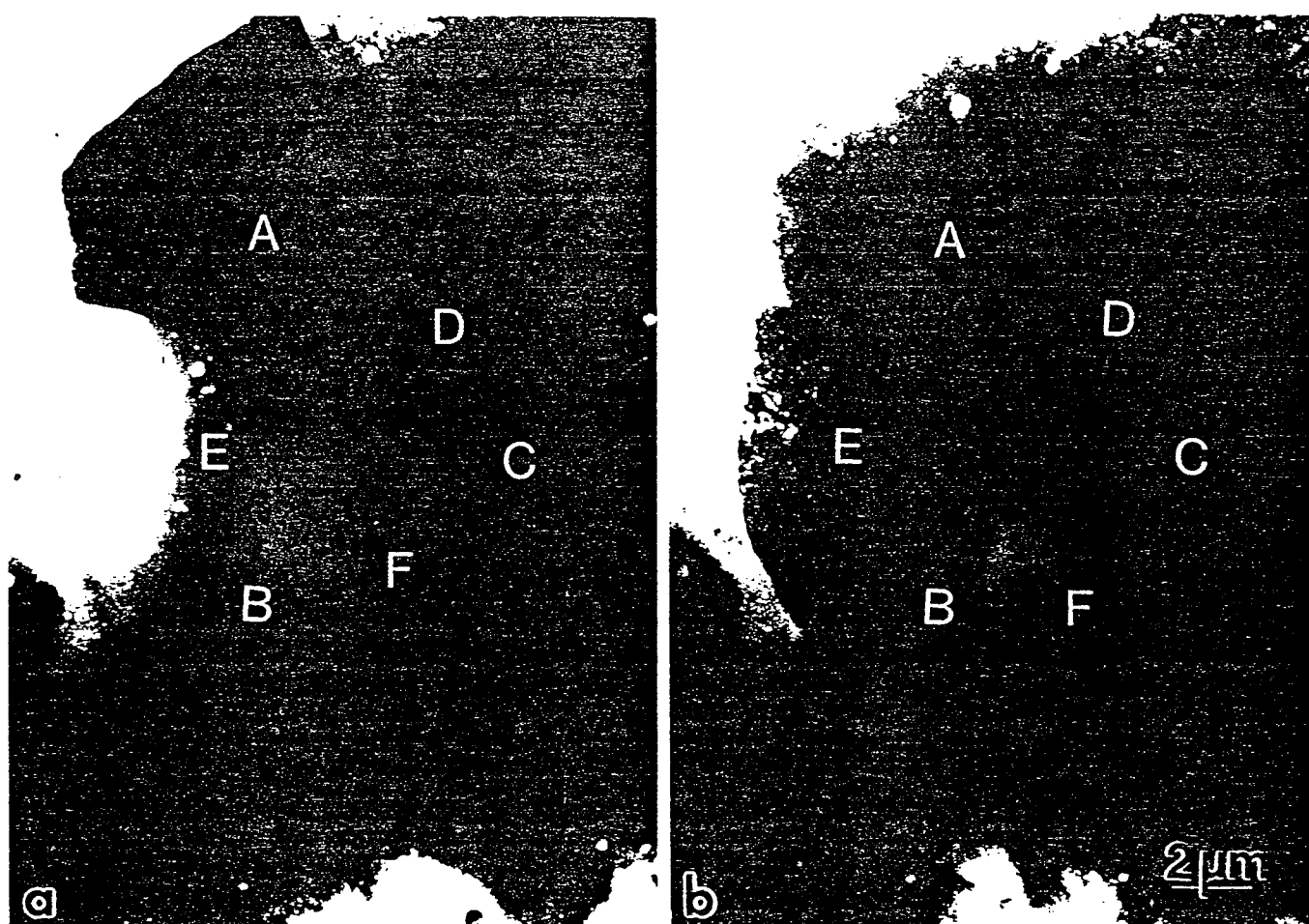


Figure 1 - First order twinning that takes place among the platelets. Bright field images before (a) and after (b) slight tilting of the sample to reveal the contrast reversal.

The two electron diffraction patterns are related to each other by twinning on  $\{110\}$  plane, although they are slightly misaligned along the  $c$ -direction. We term these twins as first order, incoherent twins between platelets. Many of the platelets have twinned domains with the crystal, such as the  $90^\circ$  domains shown in Fig. 2. These are termed second order twins with coherent twin boundaries within platelets. Twins are also observed on a nanometer scale as shown in Fig. 3. These twins form due to the necessity to accommodate the strain that is developed within each domain during the formation of  $60^\circ$  domains as the angle between  $\{110\}$  planes are  $63.5^\circ$ .<sup>5</sup>



Fig. 2 - Second order twins between domains in aragonite.

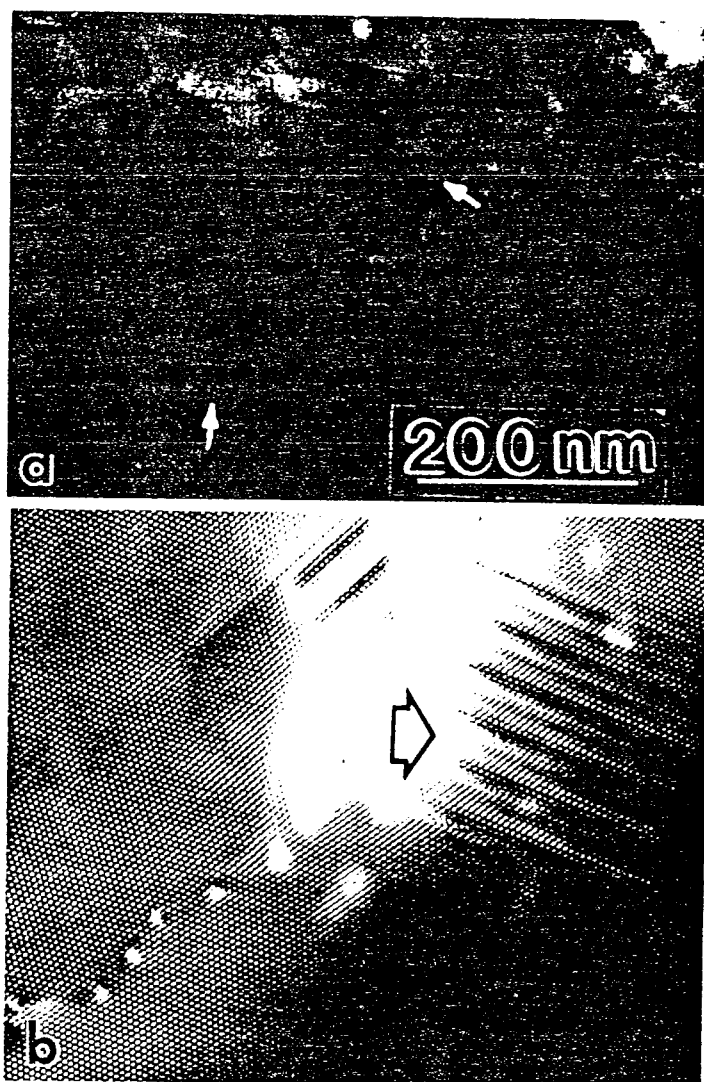


Fig 3 - Third order twins at the nanometer-scale within domains: (a) low mag. TEM image; (b) Atomic resolution image in  $[001]$  of aragonite

#### iv.2 Relation to the Crystalline Structure of the Organic Template: Superlattice Model

The results of hierarchical twinning is significant since it is the first time that in the inorganic component of a hard tissue that hierarchical order is revealed. This hierarchy occurs on a length scale ranging from millimeter to nanometer. Since the crystals nucleate and grow separately from one another (Fig. 4), the long range order has to originate from the organic template on which the  $\text{CaCO}_3$  crystals are grown. If there is a control of the nucleation and growth of the inorganic phase by the organic matrix, then it can be expected that there is crystallographic correlation between the organization of the ions in the inorganic lattice and the conformation of the active proteins within the organic matrix. In order to reveal such a crystalline conformation of the organic matrix proteins, the crystallography of the inorganic phase may be examined closely. For this, aragonite phase, looking in the [001] direction may be projected with three a-b planes rotated upon one another with a  $63.5^\circ$  rotation to mimic the three possible twin variations. For simplicity, only the position of Ca ions are illustrated in the schematic illustration in 5a. The superimposition of three such planes will generate a set of fixed positions within the new lattice, which we call a superlattice (Fig. 5b). The



Fig. 4 - Scanning electron micrograph from the growing edge of the abalone nacre showing the nuclei and their distribution.

highlighting of this superlattice (Fig. 5c)<sup>8</sup> indicates that it is a pseudo-hexagonal structure. It is tempting to say that, perhaps, the underlying organic matrix has Ca-binding proteins organized in coherence with this superlattice created by the fixed positions on the inorganic crystals as this new lattice reflect the essential characteristics of the organic template. It may indicated that, for example, a single crystalline hexagonal organic membrane structure would satisfy this requirement.

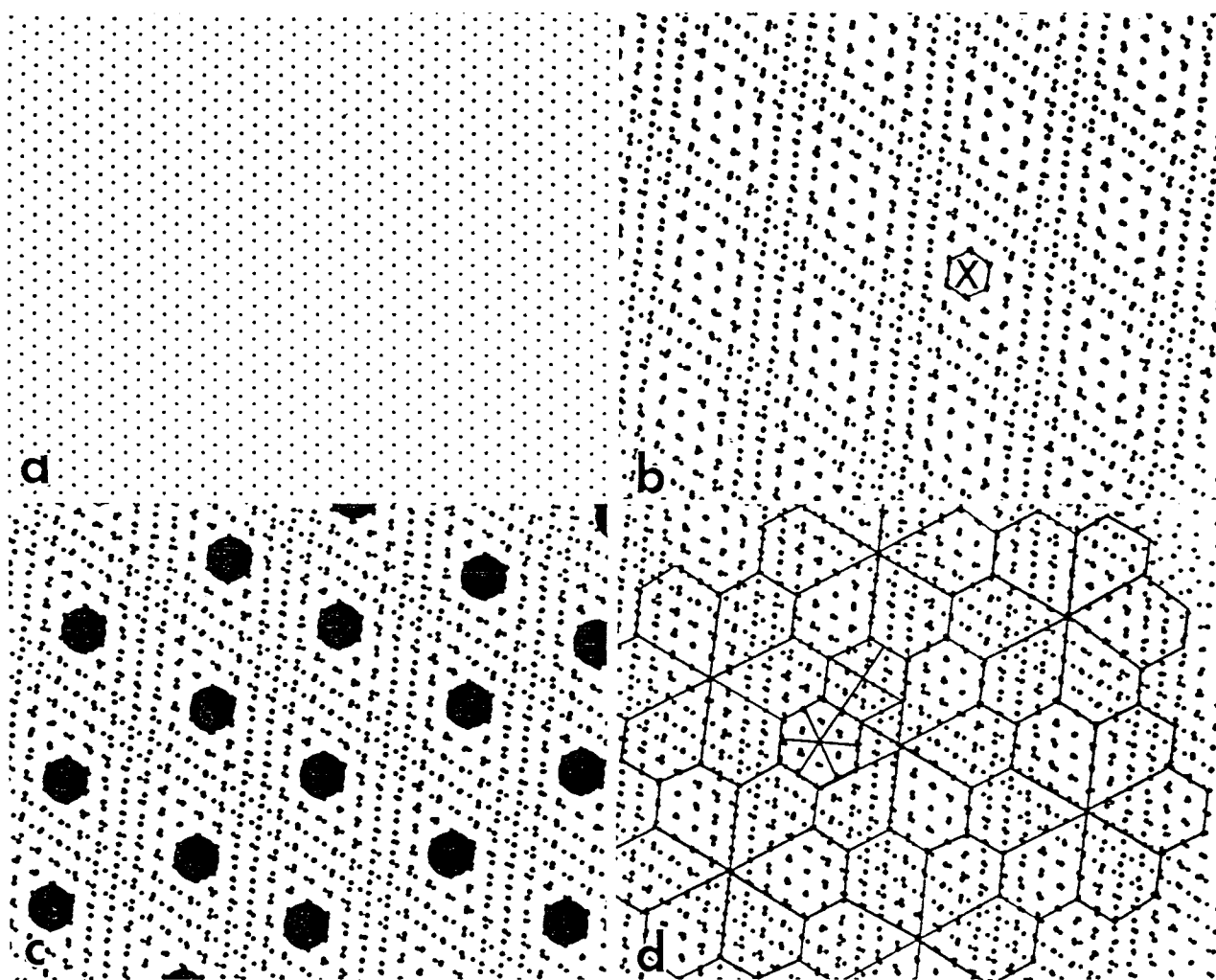


Fig. 5 - (a) Schematic illustration of the projection of the Ca ion position in the aragonite structure. (b) Superimposition of the three variants of the twins showing superlattice formation. (c) Superlattice with the fixed Ca binding positions highlighted. (d) All the geometrical and crystallographic relations of the platelets are satisfied on the superlattice structure by the model.

#### iv.4 Shape Formation of the Shell

There is evidence that the shape of the shell is related to the structure of the organic matrix. Fig. 6a and 6b show that the scaling of the growth spiral visible to the eye actually extend down to the micrometer scale. This growth pattern can be explained based upon the hierarchical superlattice model, as illustrated in Fig. 5. Therefore the spiral shape of the shell may be due to the structural constraints of the organic matrix, and, therefore, controlled by the organism.

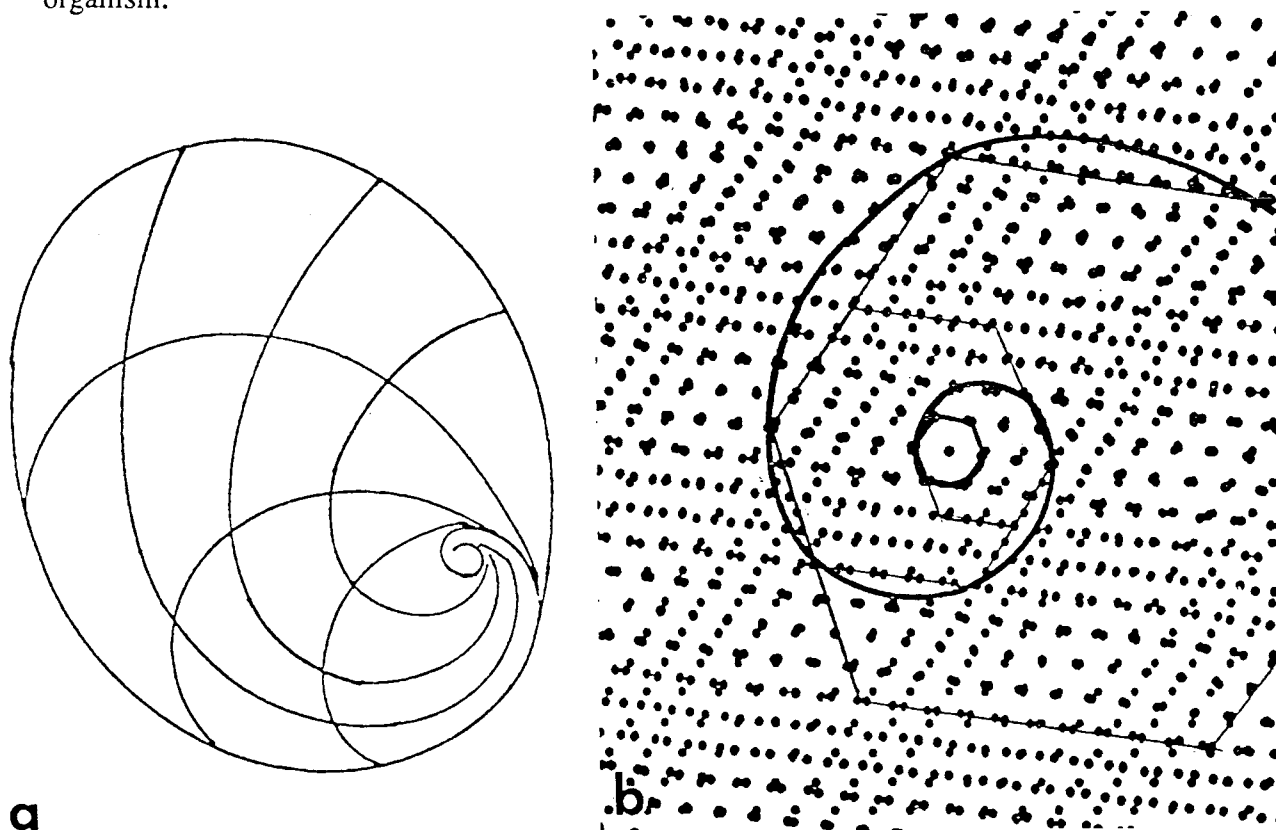


Fig. 6 - (a) Spiral description of the two-D shape of the shell; (b) generation of the spiral upon the superlattice model.

#### v. Summary and Implications of the Results in Biomimetics

The constraints of both the crystalline conformation of the organic matrix proteins and crystalline structure of the ions in the inorganic phase give rise to the hierarchical twin structure in the nacreous section of the mollusk shells. The coupling between organic and inorganic crystalline structures results in a certain morphology of the inorganic phase,

retaining the hierarchy of the defect structure in the  $\text{CaCO}_3$  phase. Based on this coupling crystallographic relationship, furthermore, even the growth pattern and shape of the overall shells can be described. There are significant implications of this result in biomimetic design of future materials. If the inorganic crystal units grow under the close scrutiny of the organic matrix, then in producing technologically significant inorganic materials (thin films, small particles, bulk or laminated composites) all the important materials structural features, such as shape, size, crystallography, and morphology, may be predicted based on the structural coupling between the organic template used and the crystal structure of the desired inorganic phase. Our future study will explore these possibilities both studying the biological composites, such as nacre, and other sections of mollusks and echinoderms, and investigating biomineralization in model synthetic systems as listed below.

## **vi. Future work**

This group's research in the biomimetic area is based on a long-range plan and, from the results of this and the previous years, the focus areas may be listed as follows:

### **i. Properties of the biological composite:**

- o- how do we measure the properties correctly (testing technique);
- o- what are the properties of the individual phases (i.e., the organic matrix and the individual inorganic platelets);
- o- what are true structures and compositions of the organic matrix, and the inorganic phase ( $\text{CaCO}_3$  + impurities + occluded organic macromolecules);
- o- what are the couplings between the functionalities of the component phases (organic matrix and inorganic phase) and their size (nanocomposite effects, mesoscopic effects, size effects);
- o- can we develop new materials fabrication techniques to mimic microarchitecture of biological composites at a smaller (nanometer) scale (we have already demonstrated in a collaborative research in another AFOSR project the laminated ceramic-metal composites, cermets, that were produced based on the nacre design at the micrometer scale).

### **ii. The mechanism of growth of nacre (not biomineralization):**

- o- what is the physical mechanism of growth of the nacre structure (how does the nacre come about);

- o- how is the spiral shape developed;
- o- are there hierarchical spiraling of the inorganic platelets that are spatially distributed and that determines the local curvature in nacre;
- o- can the overall structure of the nacre be described mathematically, based on the crystallography and morphology of the inorganic units obtained in this research;
- o- is the overall shape of the shell (curvature, morphology, size, and geometry) dictated upon the molecular structure and conformation of the organic matrix.

## ii. The organic matrix and biomineralization:

- o- what are the individual components of the organic matrix (proteins, polysaccharides, other macromolecules);
- o- composition of the organic matrix and its spatial organization;
- o- functionalities of each of the components of the organic matrix (in the nucleation, and growth of the inorganic phase, i.e., biomineralization; in giving structural and mechanical stability to the overall biocomposite; and properties;
- o- what are the Ca and/or  $\text{CO}_3^{2-}$  binding sites in the organic matrix;
- o- can individual proteins or polysaccharides self-assemble, once they are isolated; or if not, how do they self assemble (in combination of proteins, on polysaccharides,...)
- o- can biomineralization be accomplished in-vitro;
- o- can macromolecular conformation be modified, and this can be reflected on the morphology and shape, based on the crystallographic coupling between the organic matrix and inorganic crystals formed;
- o- can synthetic macromolecules, or of-the-shelf proteins, with predetermined functional groups, be used for developing substrates for biomineralization, and can the growth of inorganic components accomplished based on interface properties (the ultimate goal of biomimetics of ceramic-based composites).

Within the allocated next year and using the resources provided, we will concentrate our effort (i) property measurements, (ii) detailed characterization of the organic matrix and the inorganic phase, (iii) investigate the growth mechanism of nacre (mathematical description of the overall structure), and (iv) biomineralization studies and have plans to continue to answer many of the questions raised in this section to achieve a long term goal of producing technological materials for real life applications via either biomimicking or bioduplication.



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6. *Materials Synthesis Using Biological Processes*, MRS Proc., Vol. **174**, edited by P. C. Rieke, P. Calvert, and M. Alper (eds.) (1990); *Materials Synthesis Based on Biological Process*, MRS Proc., Vol. **218**, edited by M. Alper, P. D. Calvert, R. Frankel, P. Rieke, and D. A. Tirrell (1991); *Hierarchically Structured Materials*, MRS Proc., edited by I. A. Aksay, E. Baer, M. Sarikaya, and D. A. Tirrell (All published by Materials Research Society, Pittsburgh, 1992).
7. *Design and Processing of Materials by Biomimicking*, edited by M. Sarikaya and I. A. Aksay (American Institute of Physics, Washington, D.C., 1993).
8. M. Sarikaya and I. A. Aksay, unpublished research (1992).
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## Results on Crystallography of Nacre of Molluskan Shells (1993/94):

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University of Washington, Seattle

During the last year, we have initiated an effort to investigate the crystallography and formation of nacre structures in three different species of mollusks. The objective was to obtain detailed information on the crystallography and formation of aragonite and the development of 3-D nacre structures. In order to cover a wide range of biological structures, three mollusk species were studied: red abalone (*Haliotis Rufescens*), pearls oyster (a bivalve), and nautilus (*Nautilus pompelius*). These structures are now being compared to those of geological aragonite in which the aragonite crystal formation and morphology is significantly different. By comparing the two sets of materials, i.e., biogenic and geological, our goal is to understand the degree to which the organisms control the formation, crystallography and morphology of inorganic materials. In the following pages some of the results are presented (In the final report, a comprehensive analysis of these and the rest of the areas investigated will be presented).

**(A) Structural Investigation of  
Molluscan Nacre**

**(B) Studies of Mechanism  
of Mineralization in  
Biogenic Composite  
Ceramic/Polymer System  
and in Geological Aragonite**

**Daniel Frech and  
Mehmet Sarikaya**

## Objectives

Study of Biogenic ceramic/polymer composite material system to understand:

- (i) Mechanism of crystal formation, and controlled growth of single-crystalline aragonite tablets (in nacre) of highly specialized shape and orientation.
- (ii) Influence of polymeric (organic macromolecular) components on inorganic crystal growth in a specific biogenic ceramic/polymer composite material system, nacre.
- (iii) Special characteristics (microstructural, esp.) of biogenic crystalline phase (aragonite) in nacre, *vis a vis* its geologic counterpart.

Structural characterization of nacre, a composite formed primarily of  $\text{CaCO}_3$  (aragonite) together with small fraction of organic macromolecular component.

- (i) Investigation, via TEM, of morphology of crystalline platelets (aragonite) found in nacre, from three different molluscan organisms:

Abalone (class: *gastropoda*)

Nautilus (class: *cephalopoda*)

Pinctada (class: *bivalva*)

- (ii) Crystallographic study of aragonite platelets from nacreous portion in shells of the three molluscan organisms.

Compositional analysis and comparison, aragonite from molluscan nacre versus geologic aragonite.

- (i) Energy dispersive X-ray analyses of 3 molluscan nacre samples, and of geologic aragonite sample.

Analysis of microstructural defects, as found in biogenic aragonite versus geologic aragonite, to reveal microstructural differences which may relate to high mechanical performance of biogenic composite.

## Results

- Morphological Similarity, Aragonite Platelets in molluscan nacre from three distinct classes of organism.

- (i) All nacre samples show platelet thickness of approximately 0.5  $\mu$ m, for pinctada platelets are somewhat thicker on average.
- (ii) Platelets are generally four, five, or six sided, as viewed from (001) direction.
- (iii) Proportionality of crystalline to organic layers is consistent for all nacreous samples.

- Crystallographic Results, Molluscan Nacreous Aragonite:

- (i) Common c-axial direction
- (ii) (110) direction not well aligned between neighboring platelets.
- (iii) Neighboring subgrain domains are often twinned.

- Compositional Analysis: Differences and Similarities, Nacreous Aragonite versus Geologic Aragonite:

- Defect Structural Analyses

- (i) Twins (common) at several levels of hierarchy.
- (ii) Dislocations and stacking faults seen in geologic aragonite
- (iii) Pores seen in biogenic aragonite.

## Future Work

### A. Lessons from Biology:

Investigation of:

(1) Crystallography and Defect Structures in biogenic aragonite and geologic aragonite specifically (defects to investigate)

a) Twins

b) Stacking Faults

c) Phase Boundaries

d) Dislocations

(2) Nucleation and growth characteristics of aragonite

(a) Biogenic Aragonite:

-In "sheet" Nacre (Abalone, Nautilus)

-In Second Nacreous form (Pinctada)

(b) Geologic Aragonite

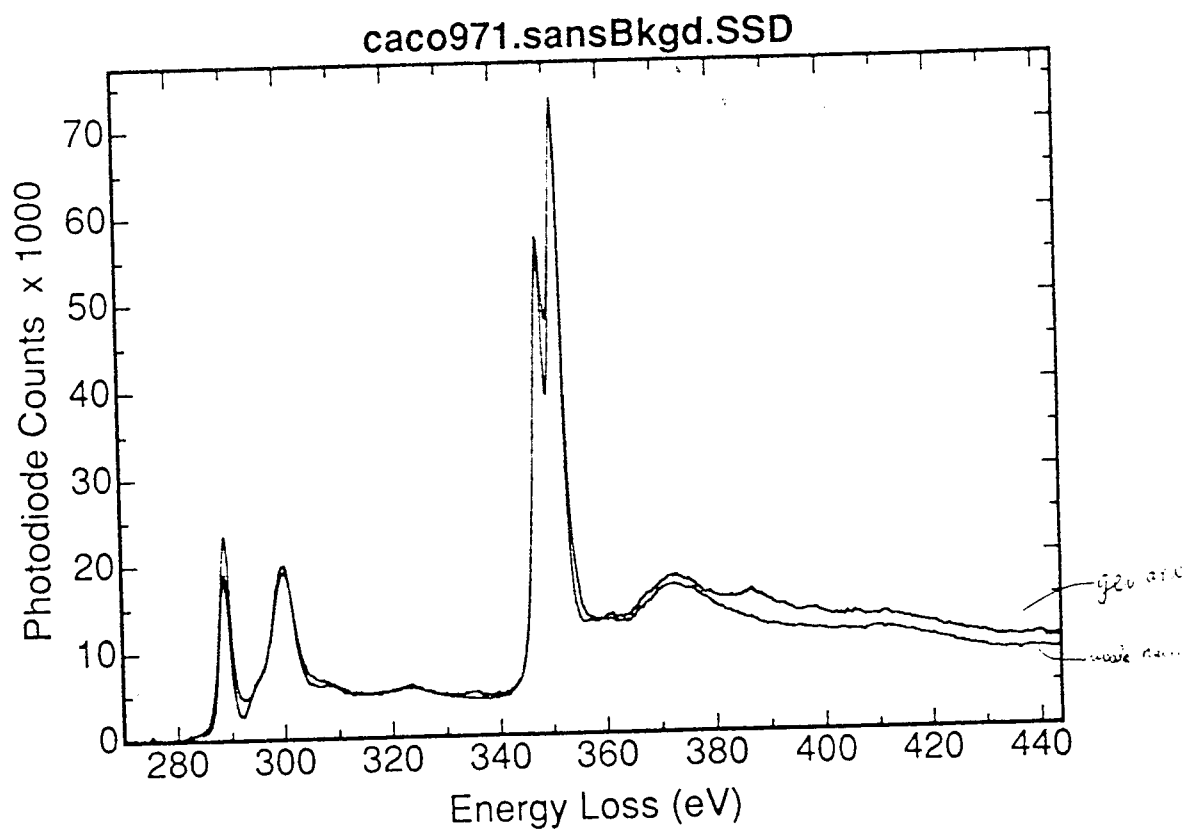
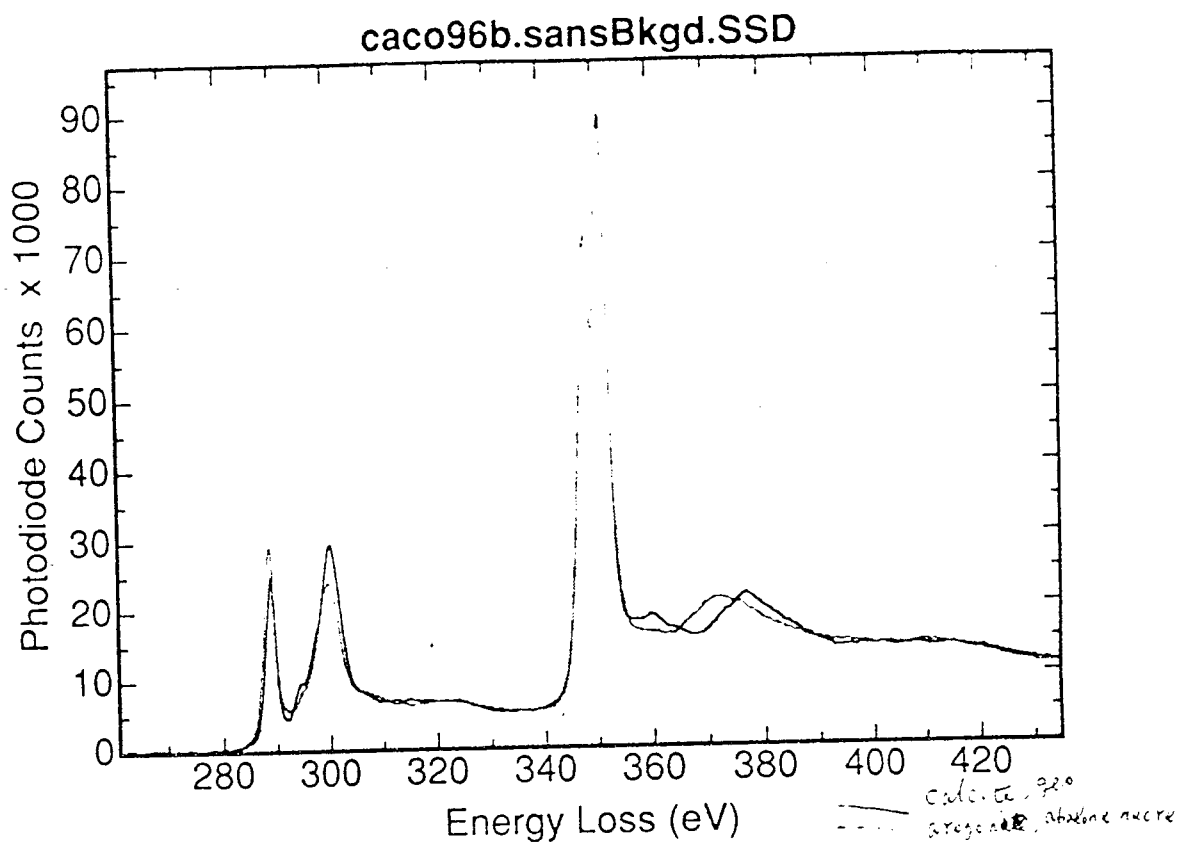
The objective is to elucidate what differences exist between biogenic and geologic aragonite, and what control organic components exert over the formation of aragonite in nacre.

(3) Interface structures and properties

(4) Correlation between microstructures of nacreous layer and prismatic layer in mollusc shell.

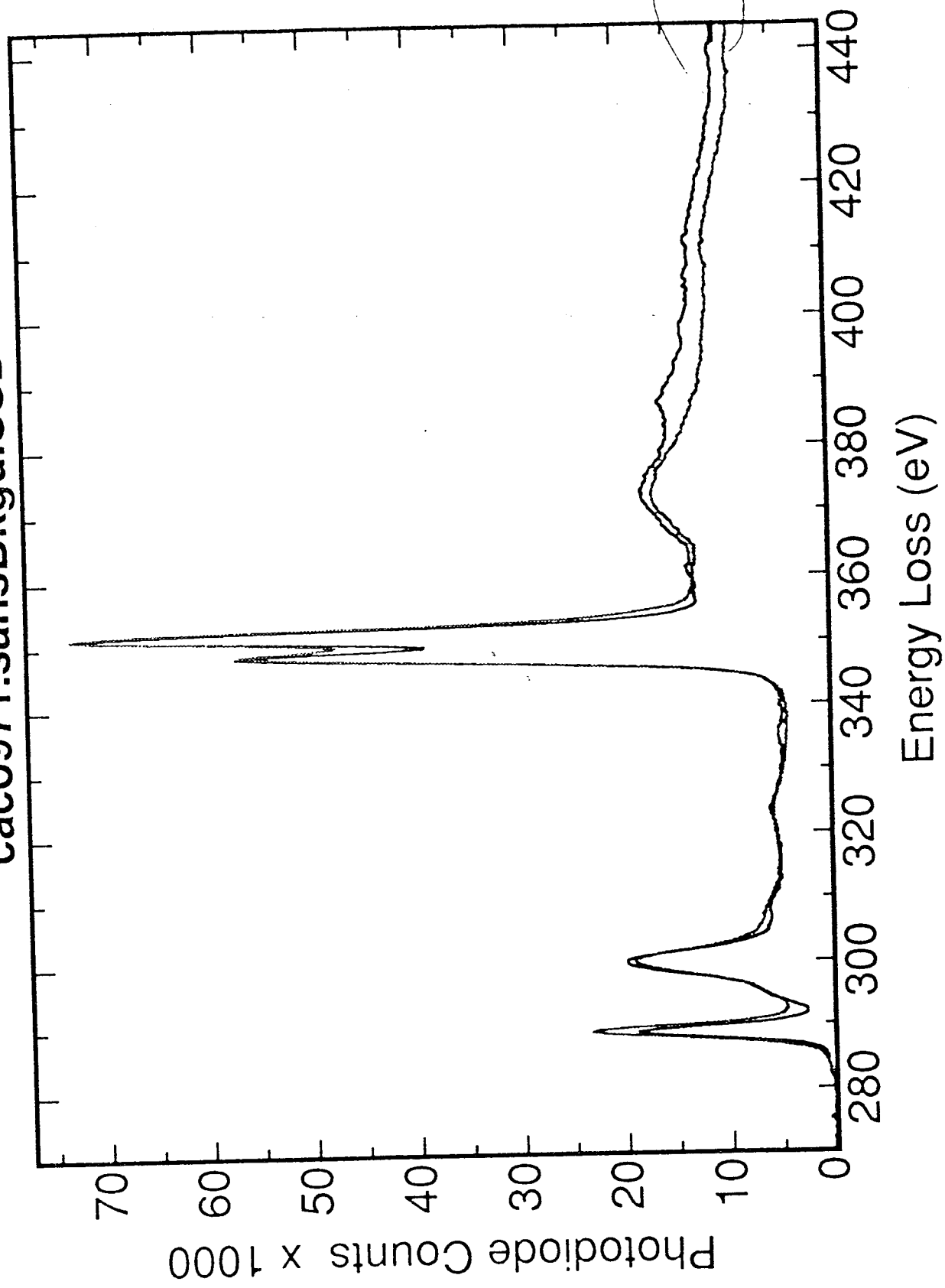
### B. Biomineralization

(1) Perform controlled experiments for aragonite and calcite formation from aqueous solutions, including in presence of organic macromolecular species.



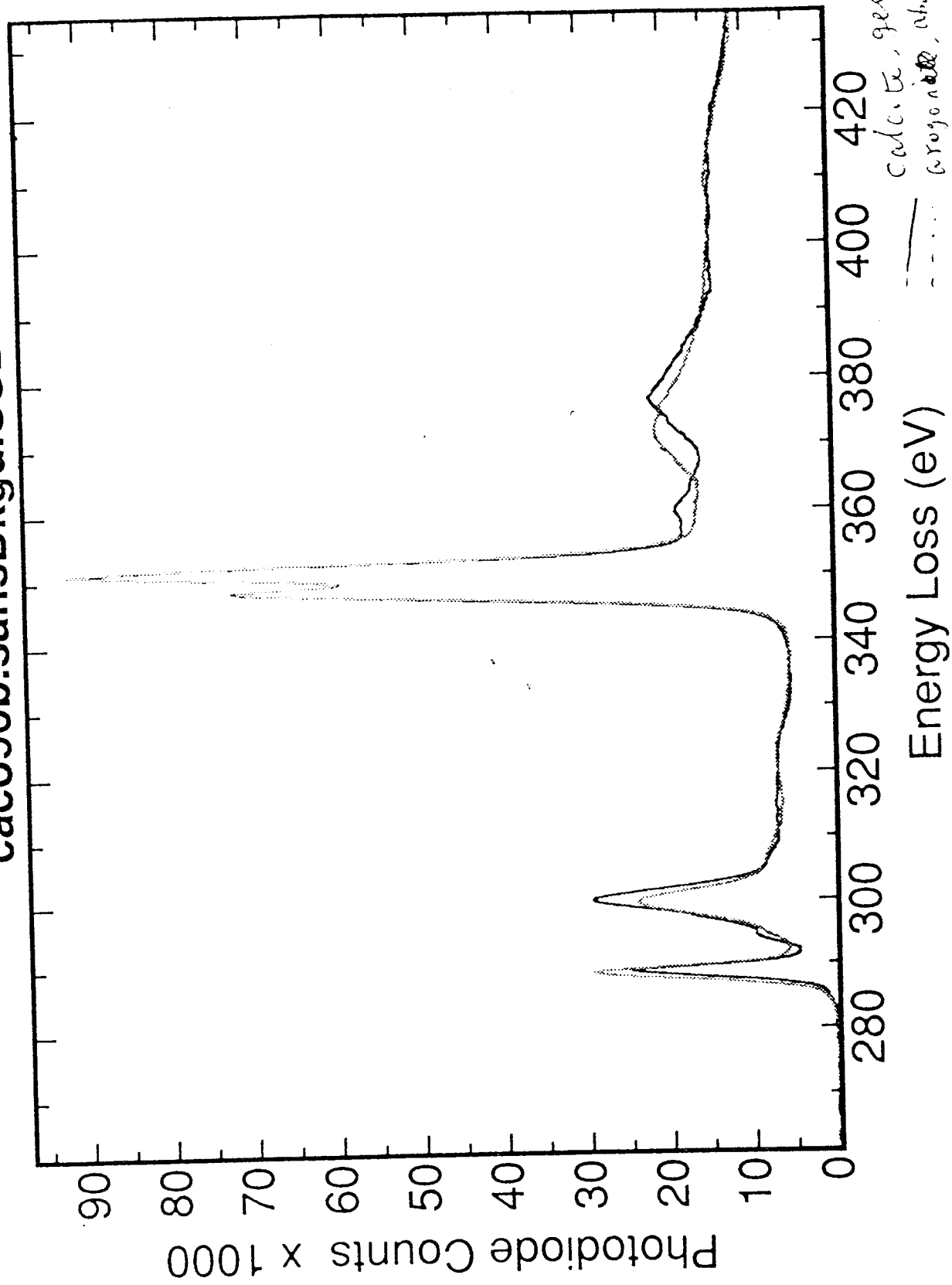
Near edge structure analysis of the electron energy loss spectra from geological and biogenic aragonite (top spectra) and calcite and aragonite from nacre (bottom figure).

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caco96b.sansBkgd.SSD



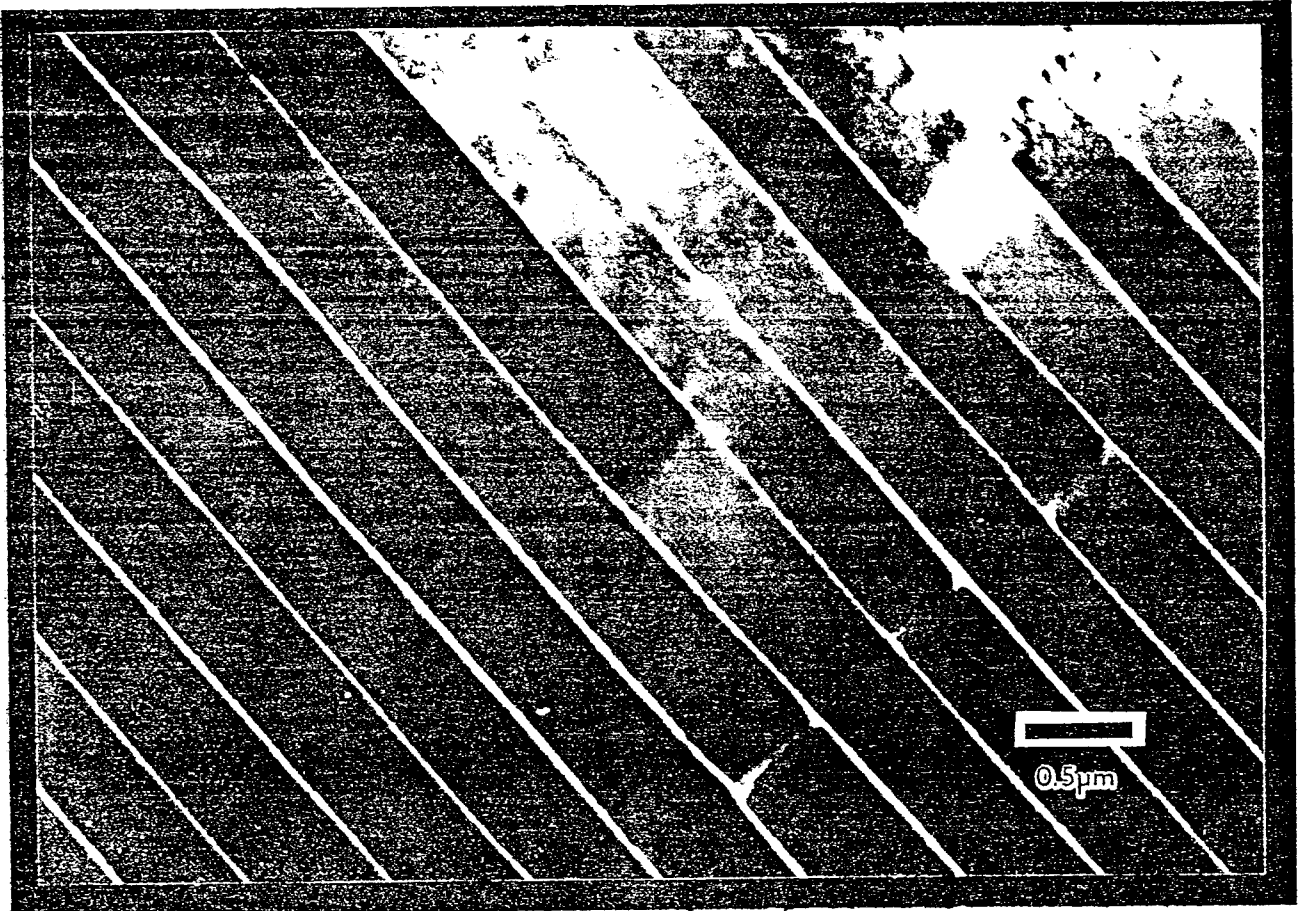


Figure (1):  
TEM Bright Field Image  
Nacre of Abalone  
Darkly tinted platelets are in strongly diffracting condition  
Average Platelet Thickness:  $0.4 \pm 0.05 \mu\text{m}$

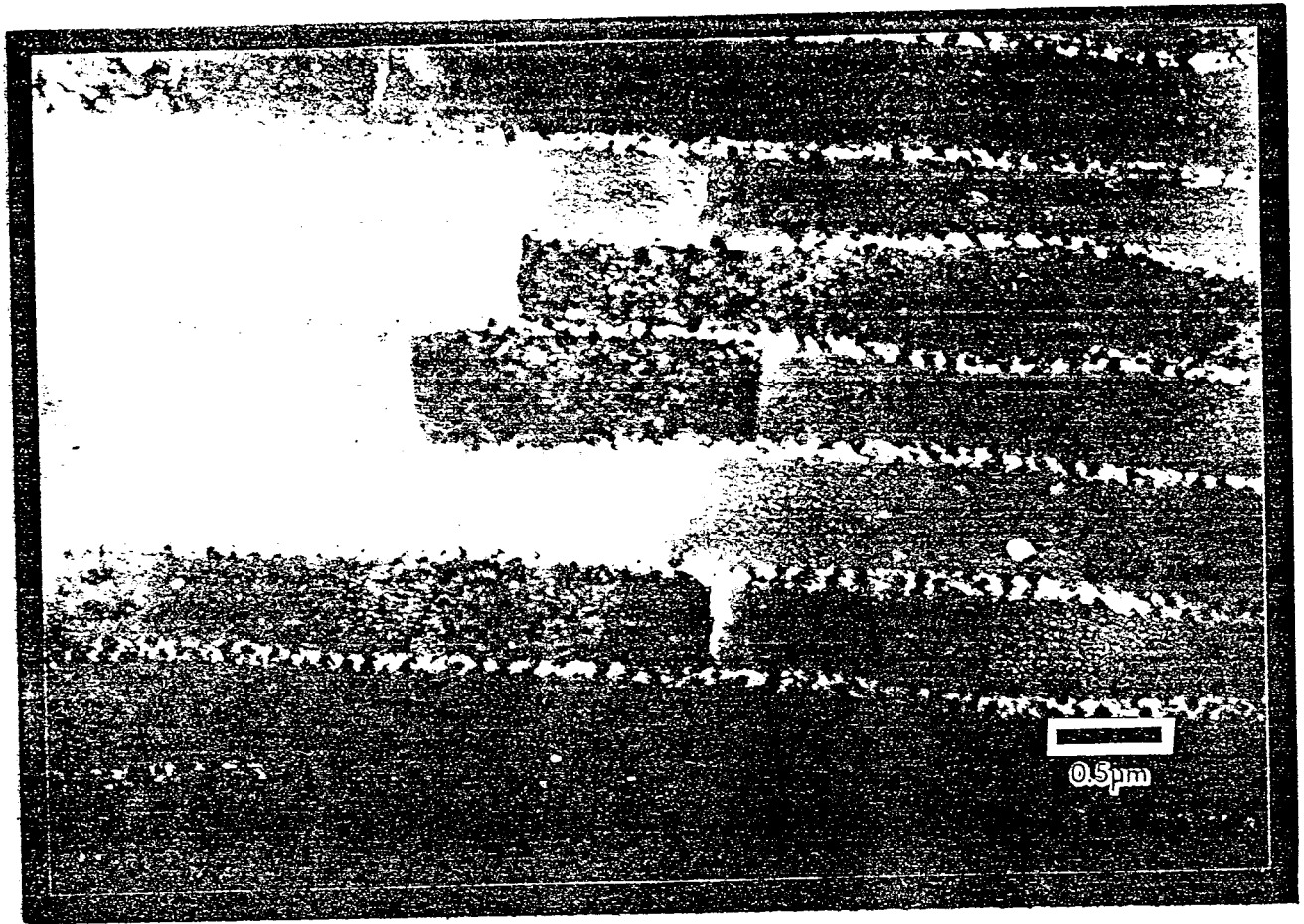


Figure (2):  
TEM Bright Field Image  
Nacre of Nautilus  
Edge-On Perspective, Aragonite Platelets  
Darkly tinted platelets are in strongly diffracting condition  
Average Platelet Thickness:  $0.5 \pm 0.05 \mu\text{m}$

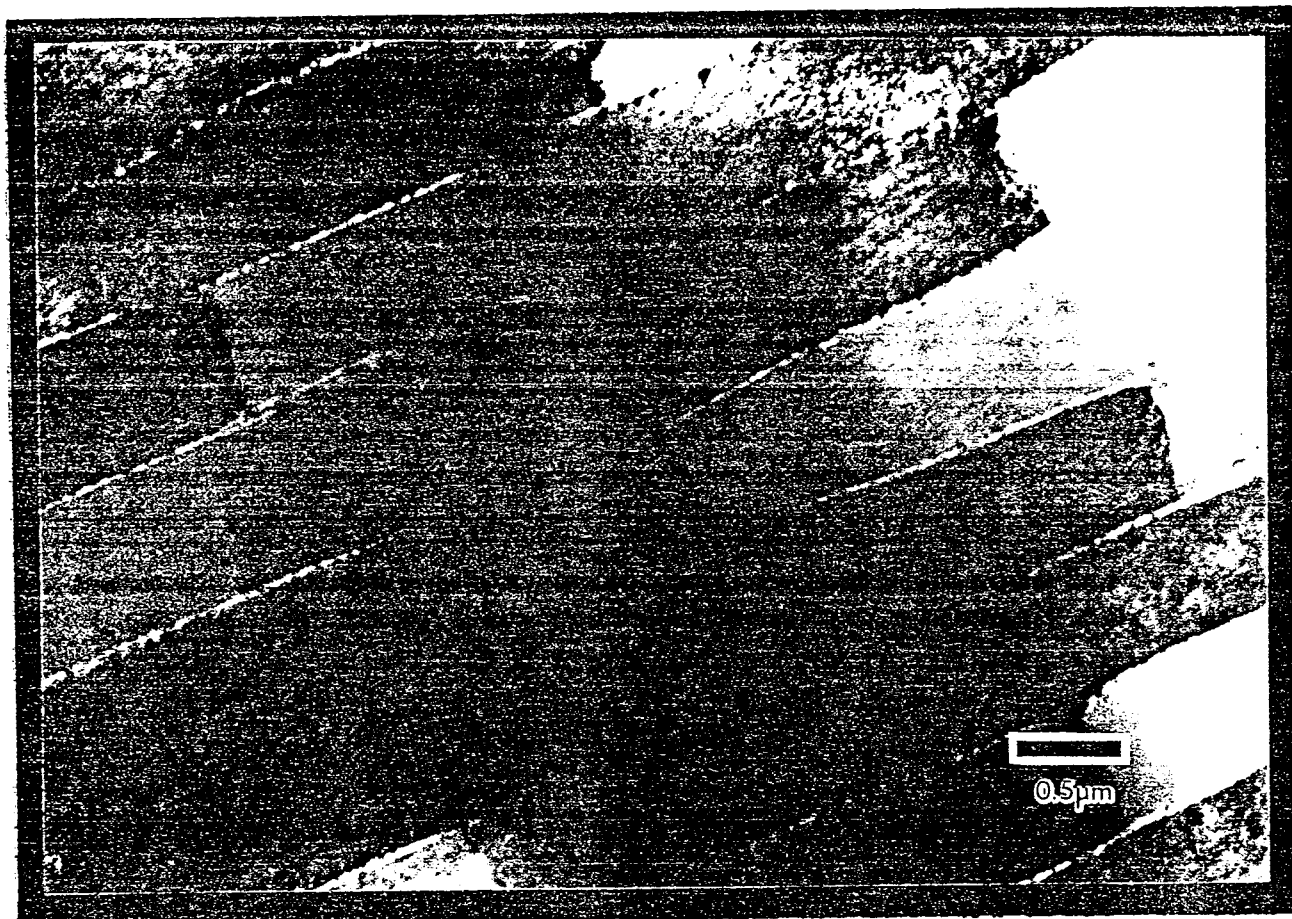


Figure (3):  
TEM Bright Field Image  
Nacre of Pinctada  
Edge-On Perspective, Aragonite Platelets  
Darkly tinted platelets are in strongly diffracting condition  
Average Platelet Thickness:  $0.7 \pm 0.05 \mu\text{m}$

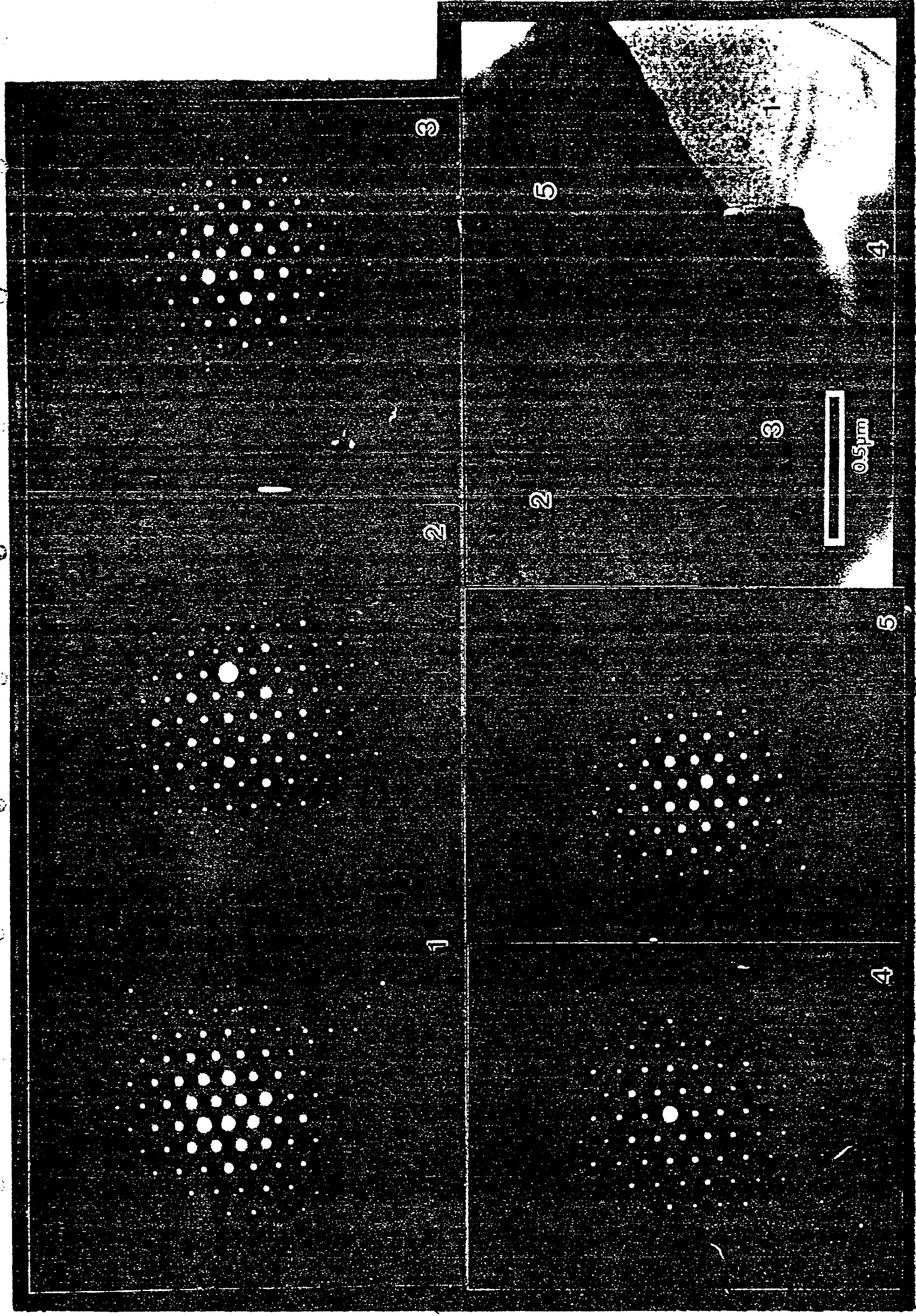
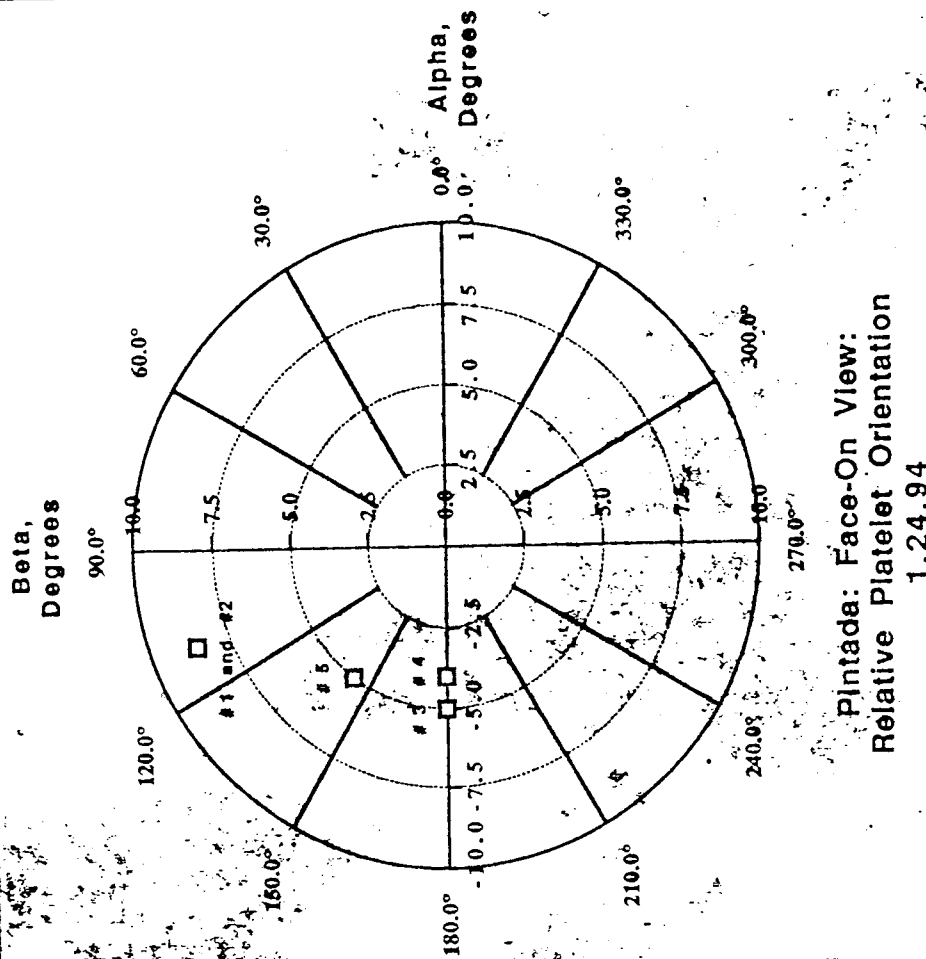
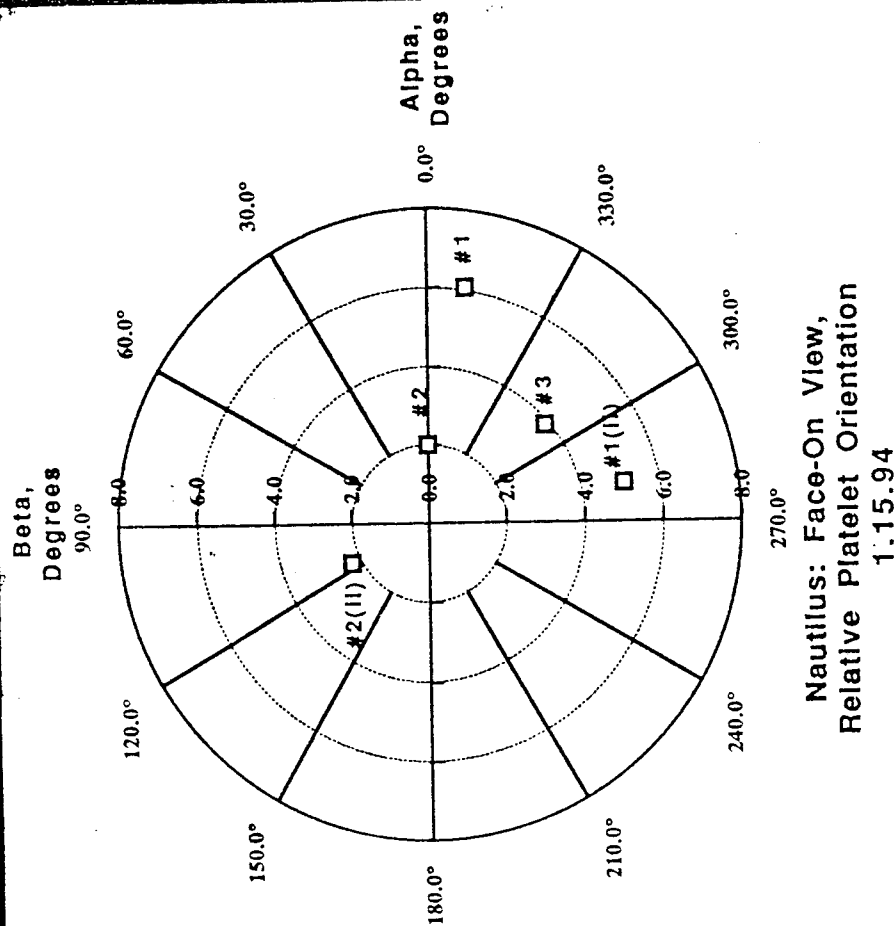


Figure 4:  
Bright Field Image, 5-Grain Junction in Nacre of Nautilus  
Face-On Perspective, Aragonite Platelets, and Diffraction Patterns for consistent zone axial  
condition taken from Respective Platelets at slightly different sample orientation



**Figure 5(a):**  
Polar Plots, Indicating Relative Alignment of [001] Direction  
for Aragonite Platelets in Nacre from Pinctada and Nautilus  
Note: The [001] axis in tablets in aragonite is nearly coincident  
for many platelets in the nacre of the three mollusks.

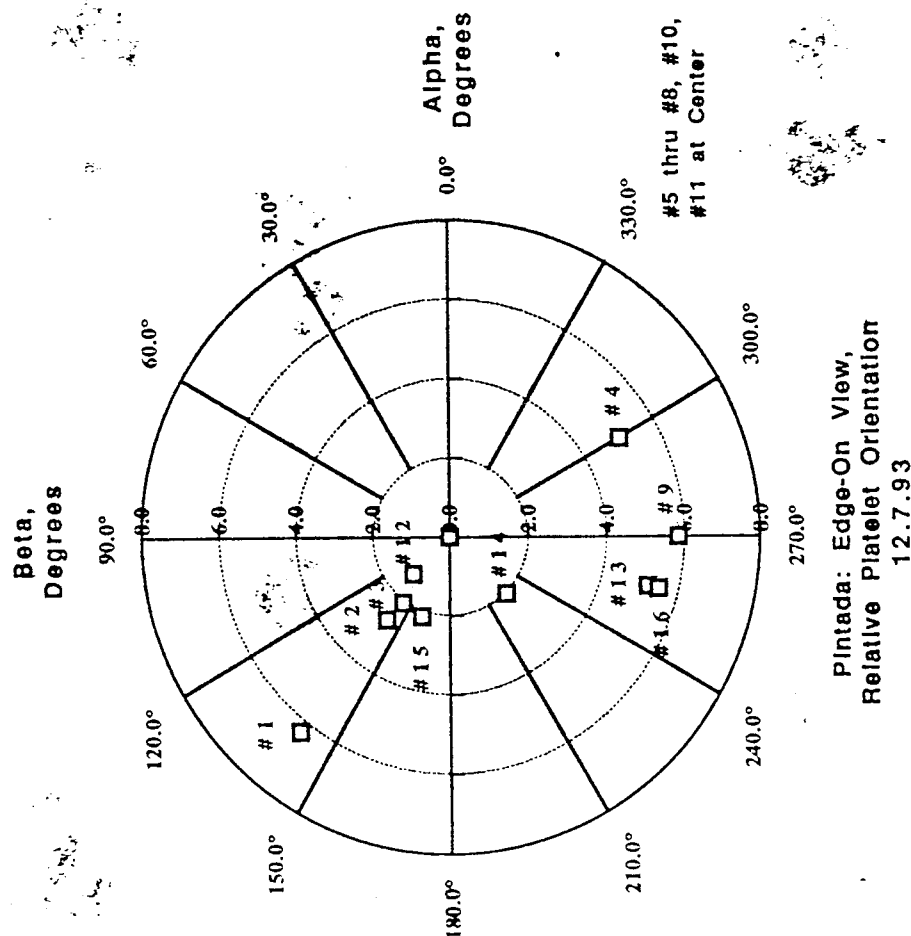
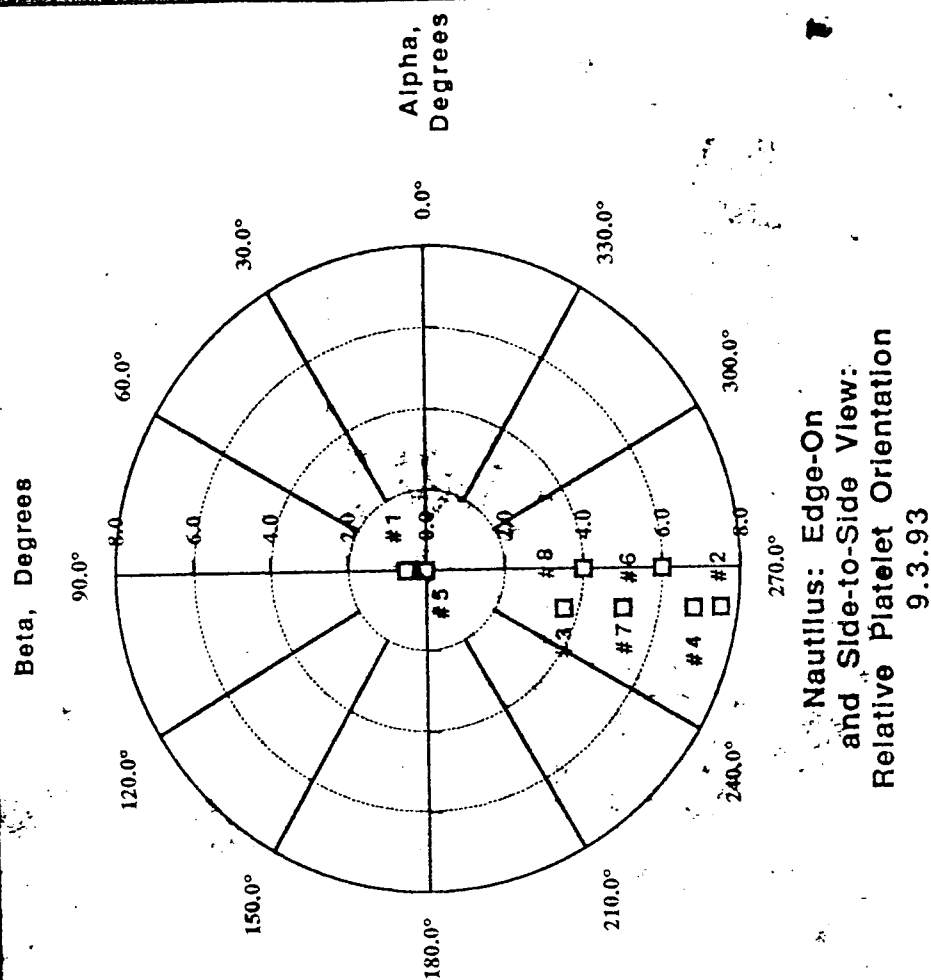
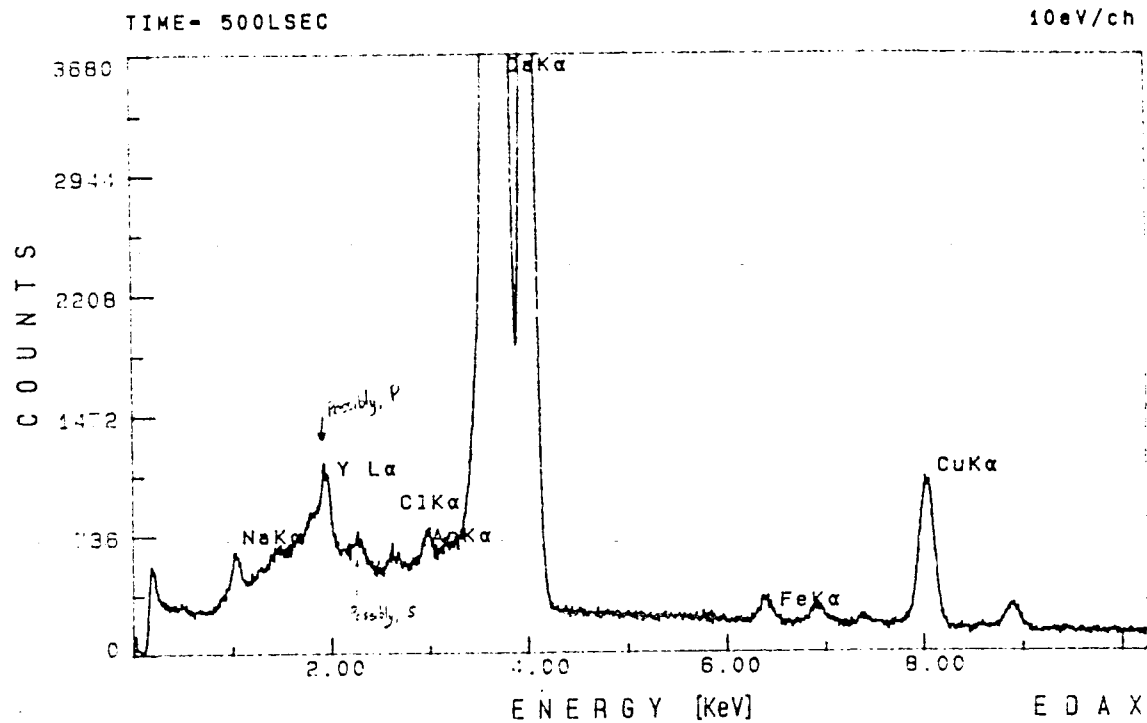


Figure 5(b):  
Polar Plots, Indicating  
Relative Alignment of (110) Direction  
for Aragonite Platelets in Nacre from Nautilus and Pinctada  
Note: The consistency of [110] alignment is much  
less than that for [001] direction in tablets of aragonite from nacre



Geological Aragonite  
Perpendicular to c-axis  
TIME= 282LSEC

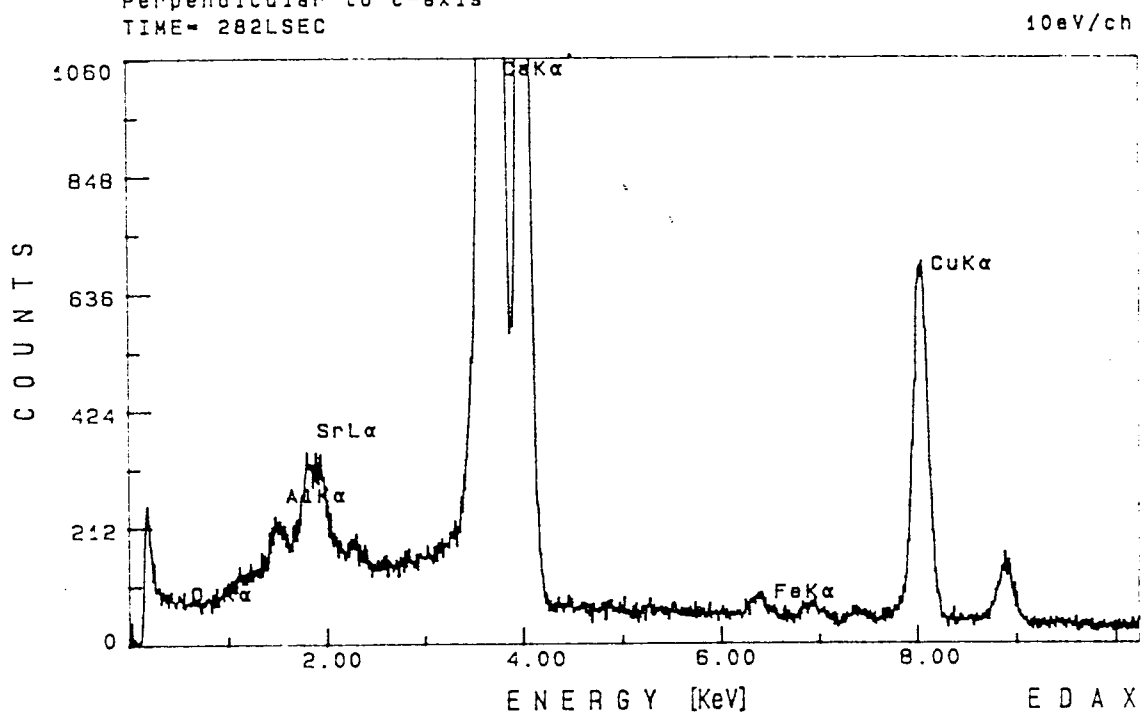


Figure 7(b):  
EDS Spectra, Recorded Using Philips 430T TEM, for:  
Molluscan Aragonite (Nautilus) and for Geologic Aragonite

Daniel Frech



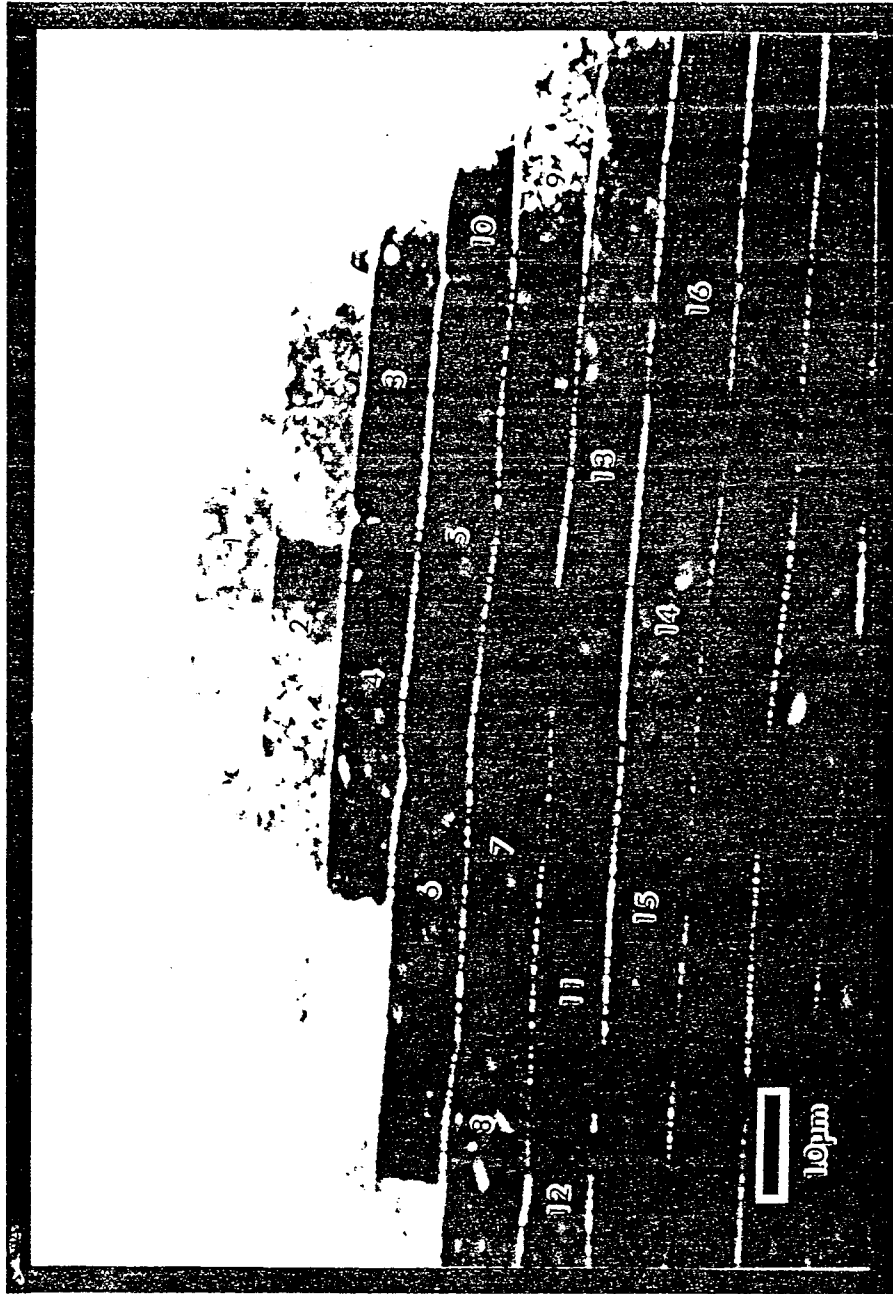


Figure (6):  
TEM BF of Region Surveyed in Pinetada Nacre (edge-on configuration)  
with specific platelets , shown in first polar plot in Figure 5 (b), numbered

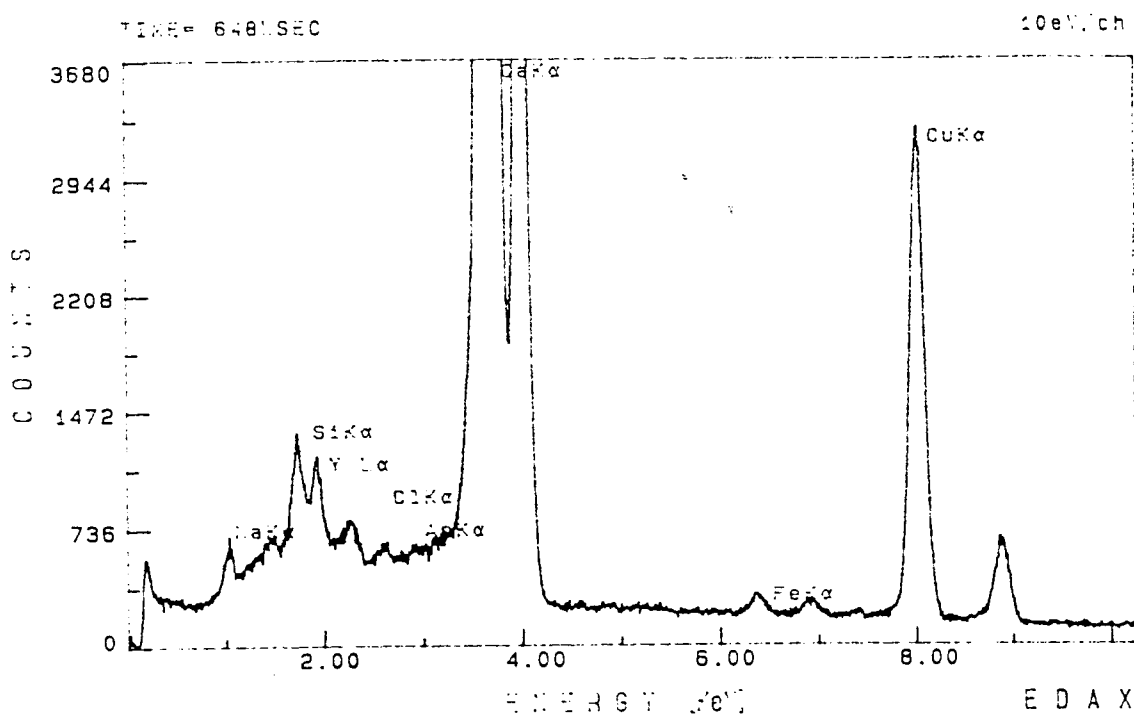
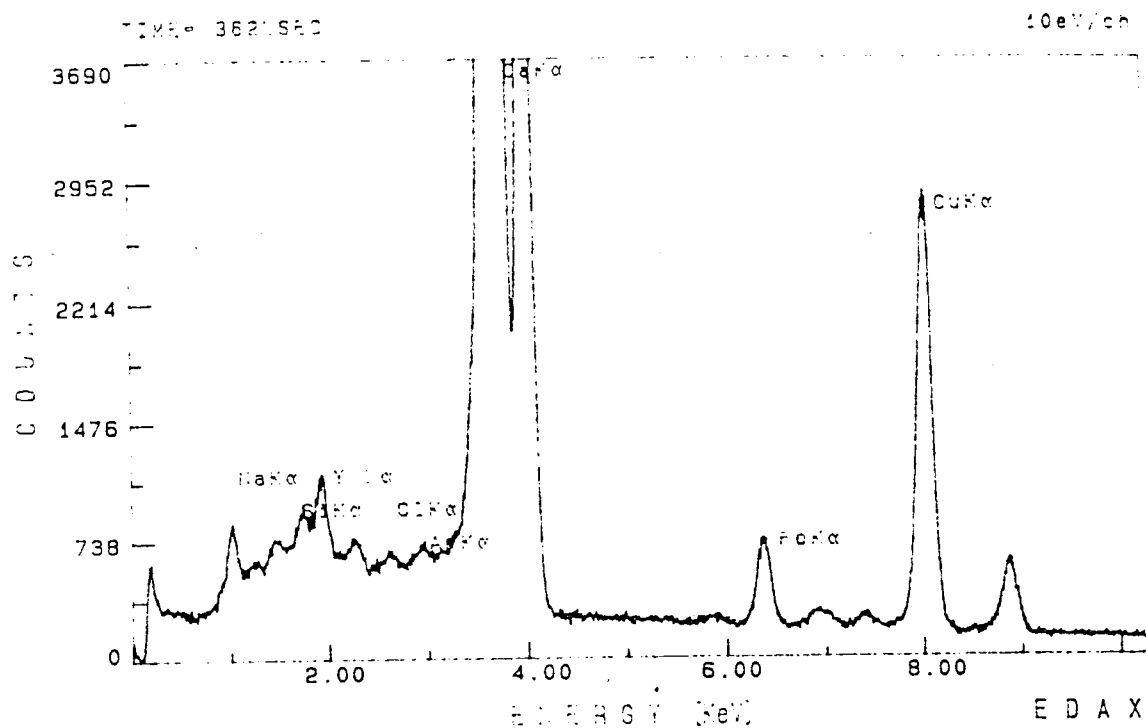
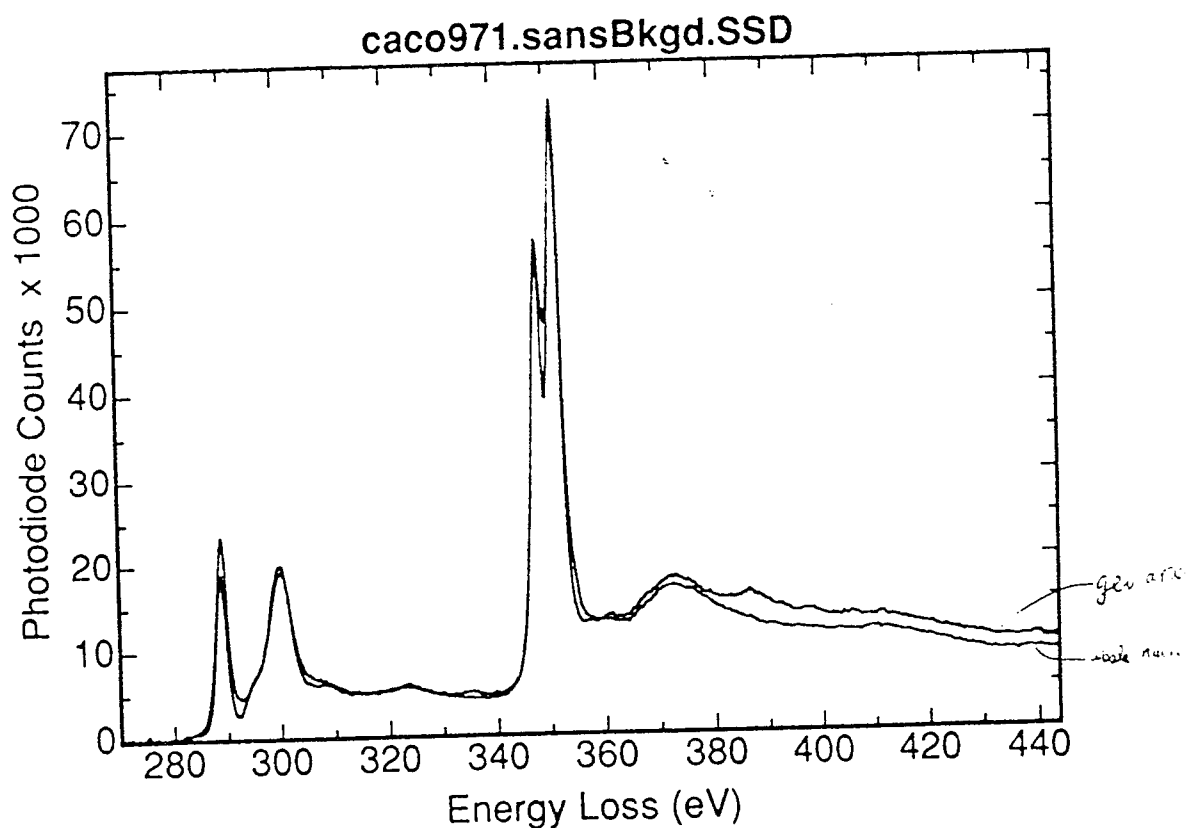
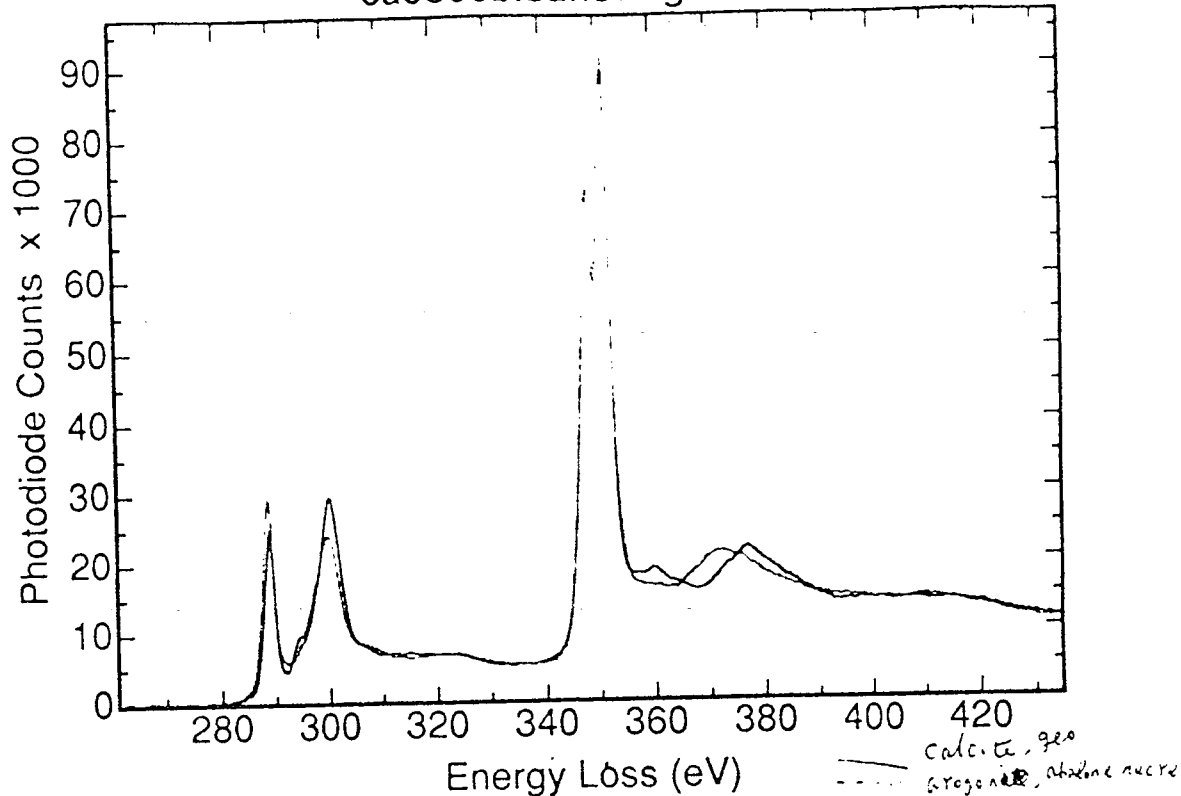


Figure 7(a):  
EDS Spectra, Recorded Using  
Philips 430T TEM, for:  
Molluscan Aragonite (Abalone and Pinctada)

Daniel Frech



Near edge structure analysis of the electron energy loss spectra from geological and biogenic aragonite (top spectra) and calcite and aragonite from nacre (bottom figure).

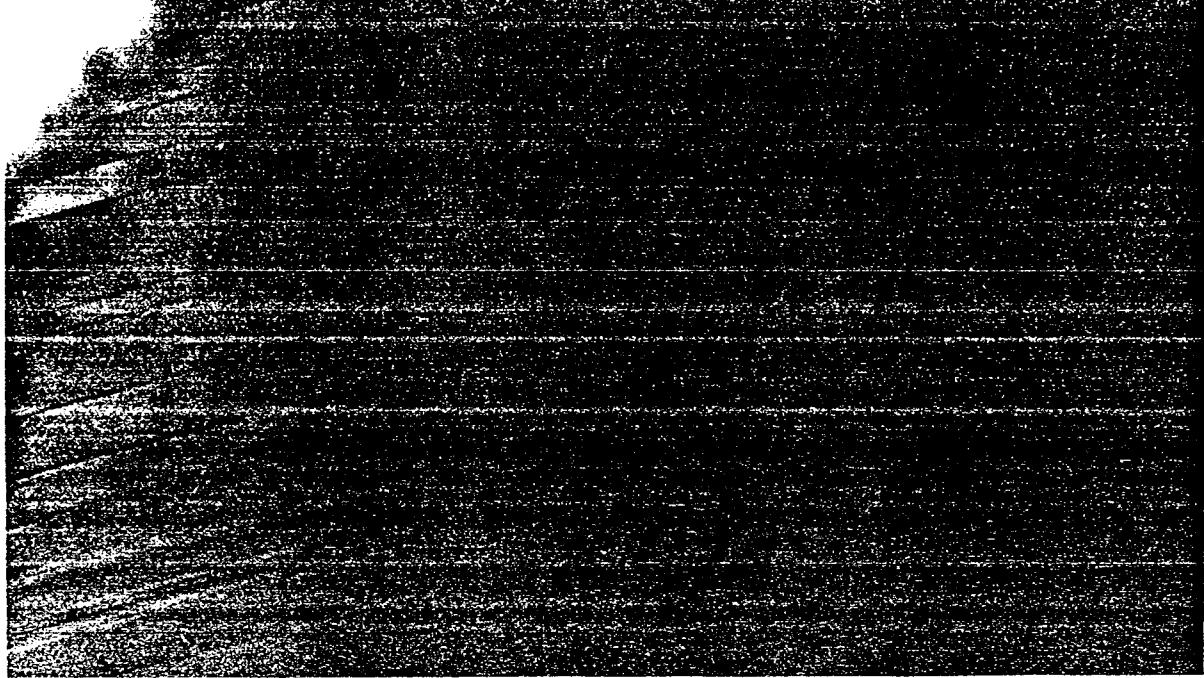


Figure (8):  
High-Magnification BF Image of Geologic Aragonite  
Features (Defect Structures) Shown Include:  
Dislocations, Stacking Faults, and Twin Domains  
Complete characterization of defect structures using techniques  
of transmission electron microscopy remains to be completed

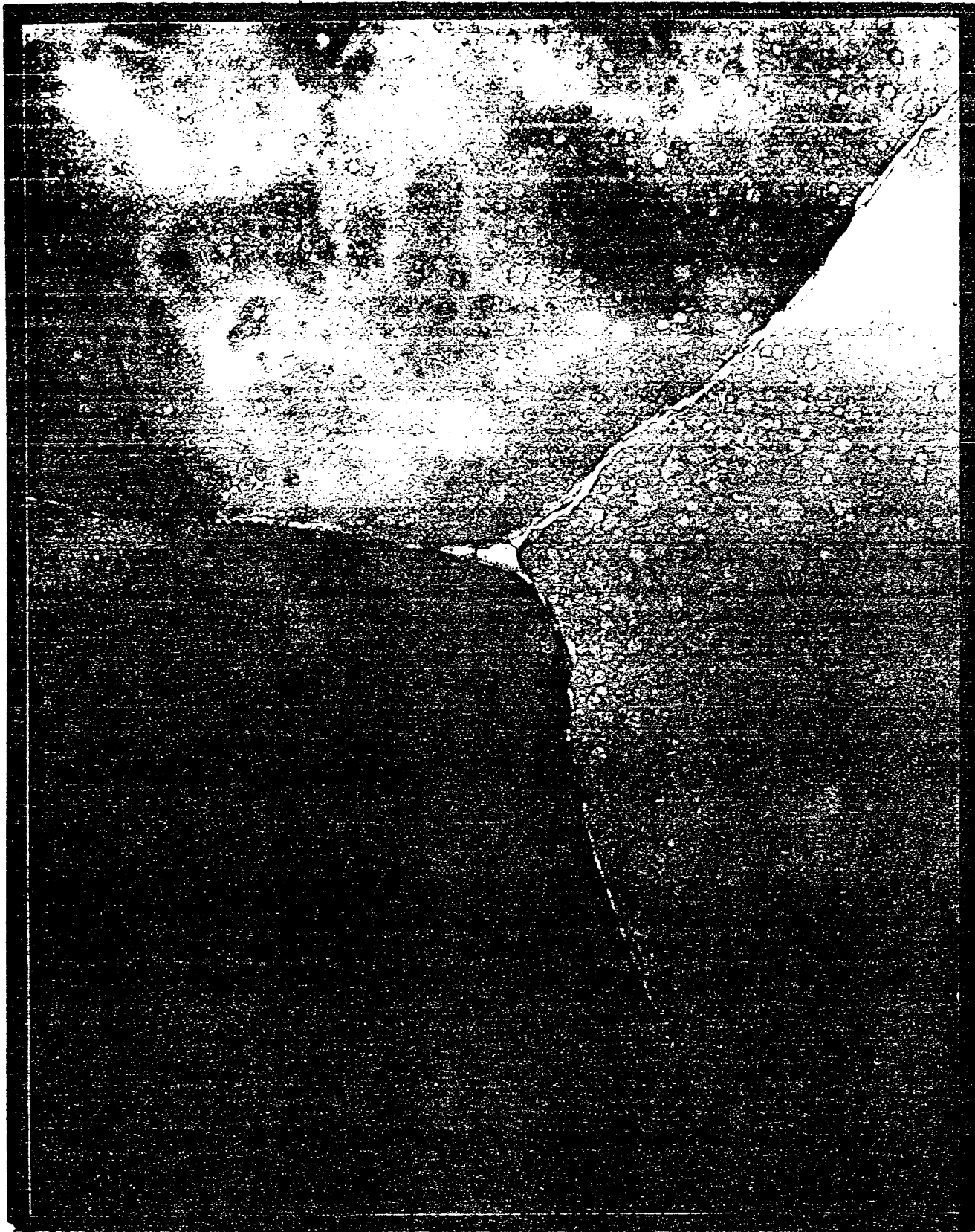


Figure (9):  
High-Magnification BF Image of Aragonite  
From Molluscan Naere  
Features (Defect Structures) Shown Include:  
Pores, Twin Domains, No Dislocations

### 3. Publications and Presentations

#### 3.1 Publications

##### Proceedings Edited:

1. *Hierarchically Structured Materials*, Proceedings of an MRS Symposium, edited by İlhan A. Aksay, E. Baer, M. Sarikaya, and D. A. Tirrell, Vol. 228, 1-450 (Materials Research Society, Pittsburgh, PA, 1992).
2. **Resolution in Microscopy**, Special Issue of *Ultramicroscopy*, 47, [1-3] 1-306 (Elsevier, Amsterdam, 1992); edited by M. Sarikaya.
3. *Determination of Nanoscale Physical Properties of Materials by Microscopy and Spectroscopy*, Proc. of MRS Symposium, edited by M. Sarikaya, K. Wickramasinghe, and M. Isaacson, Vol. 332 (Materials Research Society, Pittsburgh, PA, 1994) (80 papers and 700 pages).

##### Published Manuscripts and Those in the Works:

1. M. Sarikaya and I. A. Aksay, "Nacre of Abalone Shell: A Natural Multifunctional Nanolaminated Ceramic-Polymer Composite Material," Chapter 1, in RESULTS AND PROBLEMS IN CELL DIFFERENTIATION, Vol. 19: *Structure, Cellular Synthesis, and Assembly of Biopolymers*, edited by Steven Case (Springer and Verlag, Amsterdam, 1992) pp. 1-25.
2. I. A. Aksay and M. Sarikaya, "Bioinspired Processing of Composite Materials," in *Ceramics: Toward the 21st Century, Centennial International Symposium*, edited by N. Soga and A. Kato (Ceramic Society of Japan, Tokyo, 1991) pp. 136-149.
3. J. Liu, M. Sarikaya, and I. A. Aksay, "Hierarchical twin Structures in the Nacre of Red Abalone Shell," *49th Ann. Meeting of Electron Microscopy Society of America*, edited by G. W. Bailey (San Francisco Press, San Francisco, 1991) pp. 848-849.
4. M. Sarikaya and I. A. Aksay, "Synthetic and Biological Nanocomposites," *Proc. of 5th Intl. Conf. on Ultrastructure Processing*, edited by L. L. Hench and J. K. West (Wiley, New York, 1991) pp. 543-550.
5. M. Sarikaya, "Evolution of Resolution in Microscopy," *Ultramicroscopy*, **47** [1-3] 1-16 (1992).
6. N. B. Pellerin, G. L. Graff, D. R. Treadwell, J. T. Staley, and I. A. Aksay, "Alginate as a Ceramic Processing Aid," *Biomimetics*, **1** [2] 119-130 (1992).

7. T. Ren, N. B. Pellerin, G. L. Graff, I. A. Aksay, and J. T. Staley, "Dispersion of Small Ceramic Particles ( $\text{Al}_2\text{O}_3$ ) with *Azotobacter vinelandii*," *Appl. Environmental Microbiol.*, **58** [9] 3130-3135 (1992).
8. K. Gunnison, M. Sarikaya, J. Liu, and I. A. Aksay, "Structure-Mechanical Property Relationships in a Biological Ceramic-Polymer Composite: Nacre," in *Proc. of Hierarchically Structure Materials*, Symposium-Z, edited by I. A. Aksay, E. Baer, M. Sarikaya, and D. Tirrell (Materials Research Society, Pittsburgh, PA, 1992) pp. 171-184.
9. J. Liu, M. Sarikaya, and I. A. Aksay, "A Hierarchically Structured Model Composite: A TEM Study of Hard Tissue of Red Abalone Shell," in *Proc. of Hierarchically Structure Materials*, Symposium-Z, edited by I. A. Aksay, E. Baer, M. Sarikaya, and D. Tirrell (Materials Research Society, Pittsburgh, PA, 1992) pp. 9-18.
10. M. Sarikaya and I. A. Aksay, "Imaging of Hierarchically Structured Materials," in *Proc. of Hierarchically Structure Materials*, Symposium-Z, edited by I. A. Aksay, E. Baer, M. Sarikaya, and D. Tirrell (Materials Research Society, Pittsburgh, PA, 1992) pp. 293-308.
11. M. Sarikaya, "Biomimetic Materials: An Introduction," 50th Annual Meeting of Electron Microscopy Society of America, edited by G. W. Bailey, J. Bentley, and J. A. Small (San Francisco Press, San Francisco, CA, 1992) pp. 1020-1021.
12. J. Liu, K. E. Gunnison, M. Sarikaya, "A TEM Study of Interface between Organic Matrix and Aragonite in a Biological Hard Tissue, Nacre," 50th Annual Meeting of Electron Microscopy Society of America, edited by G. W. Bailey, J. Bentley, and J. A. Small (San Francisco Press, San Francisco, CA, 1992) pp. 1024-1025.
13. M. Sarikaya, "Tailoring Microstructures of Materials via Biomimetics," *Proc. 51st Ann. Meeting of Microscopy Society of America*, edited by G. W. Bailey and C. L. Reider (San Francisco Press, San Francisco, CA, 1993) pp. 500-501.
14. M. Sarikaya and I. A. Aksay, "An Introduction to Biomimetics: A Structural Viewpoint," in *Microstructure of Materials*, edited by K. M. Krishnan (San Francisco Press, San Francisco, CA, 1993) pp. 141-148.
15. M. Sarikaya, "An Introduction to Biomimetics: Structural Viewpoint," *Microsc. Res. Tech.*, **27** [5] 361-375 (1994).
16. R. Humbert, M. Sarikaya, I. A. Aksay, and C. E. Furlong, "Characterization of Organic Nucleator and Framework Macromolecules in Mollusk Shells," in *Proceedings of Materials Research Society Symposium*, Vol. 330, entitled: *Biomolecular Materials by Design*, edited by: H. Bayley, D. Kaplan, M. Navia, and M. Alper (Materials Research Society, Pittsburgh, PA, 1993).

17. M. Sarikaya, "Biological Composites: Ultimate Self-Assembled Materials," Proceedings of XIIIth International Congress of Electron Microscopy, ICEM 13, Paris, Vol. 3B: Applications in Biological Sciences (Les éditions de Physique, Paris, France, 1994) pp. 889-890.
18. M. Sarikaya, "Organic-Inorganic Interfaces In Layered Biological Composites," Proceedings of 52nd Microscopy Society of America, J. Bailey (ed.) (San Francisco Press, San Francisco, CA, 1994) pp. .
19. M. Sarikaya, J. T. Staley, and C. E. Furlong, "Nanodesigning and Properties of Biological Composites," Proc. American Society of Mechanical Engineers, Chicago, IL, November 6-8, 1994 (to be published).
20. M. Sarikaya, "Nanodesign and Properties of a Biocomposite for Biomimetics," Proc. of *Segundo Congreso Mexicano de Microscopi Electronica*, edited by F. Gasga (1994) pp. SP2.
21. M. Sarikaya, J. Liu, and I. A. Aksay, "Nacre of Abalone: Its Mechanical Properties, Morphology, Crystallography, and Formation," in: *Biomimetics: Design and Processing of Materials*, Edited by M. Sarikaya and I. A. Aksay (American Institutes of Physics, New York, 1994).
22. R. Humbert, M. Sarikaya, and C. E. Furlong, "Layered Aragonitic Particles from Mollusk Shell Extracts," Submitted to *Nature* (July 1994).
23. M. Sarikaya and J. Liu, "Mechanism of Formation of Nacre in Mullusks," to be submitted to *Nature* (Autumn, 1994).
24. M. Sarikaya and D. Frech, "Comparison of Crystallography of Biogenic and Geological Aragonite," to be submitted to *Acta. Cryst.* (Autumn, 1994).
25. S. F. Hashemifar, J. T. Staley, and M. Sarikaya, "The Degree of Environmental Damage in the Use of Synthetic and Biological Polymers in Ceramic Processing," to be submitted to *Biomimetics* (Fall 1994).
26. M. Sarikaya, "Biomimetic Ceramics," in: *Bioceramics*, edited by James Shackelford (Trans. Tech. Publ, Ltd., Lousanne, Switzerland, 1995).



**Thesis:**

## Completed:

1. K. E. Gunnison, "Structure-Mechanical property Relationships in a Biological Ceramic-Polymer Composite: Nacre," M.S. thesis (June 1991, University of Washington, 1991).
2. G. L. Graff, "Biopolymers as Aids in Ceramic Processing," M.S. Thesis (June 1991, University of Washington, 1991).
3. Sima F. Hashemifar, "The Use of Polymers in Ceramic Processing: A Comparison of Synthetic and Biopolymers for Safety Concerns," M.S. Thesis (June 1994).
4. Tao Ren, "Magnetosome Structure in Magnetite Formation in Magnetotactice Bacteria" M.S. Thesis (September, 1994).

## Continuing:

1. Daniel Frech, "Crystallography and Formation of Biogenic and Geological Calcite and Biomineralization," Ph.D. Thesis (started Fall 1992).
2. Benjamin Shapiro, "Mechanical Properties of Mollusk Shells: Model for Biomimetics of Impact Resistant Materials," M.S. Thesis (Started Fall 1994).
3. Sima F. Hashemifar, "In-Situ Processing of Ceramics in the Presence of Bacteria: Environmentally-benign Processing of Materials" Ph.D. (started Fall 1994).

### 3.2 Presentations: Invited Talks, Conferences, Workshops

#### a. Invited Talks:

1. M. Sarikaya and Ilhan A. Aksay, "Electron-Optical Characterization of Hierarchically Structured Materials," Materials Research Society Fall Meeting, Boston, MA, Dec. 2-6, 1991
2. M. Sarikaya, J. Liu, and I. A. Aksay, "Hierarchical Structures of Seashells and Exoskeletons for Biomimetics," Mater. Res. Soc. Fall Meeting, Boston, Dec. 2-6, 1991.
3. Ilhan A. Aksay and M. Sarikaya, "Processing of Synthetic Hierarchically Structured Ceramic-Based Materials," Mater. Res. Soc. Fall Meeting, Boston, MA, Dec. 2-6, 1991.
4. James T. Staley, M. Sarikaya, and I. A. Aksay, "Biomimicking with Organisms," Materials Research Society Fall Meeting, Boston, MA, Dec. 2-6, 1991.
5. M. Sarikaya, J. Liu, and I. A. Aksay, "Crystallography of the Hard Tissue in Nacre of Abalone," 4th Intl. Conf. on Miner. Tissues, Coronado Peninsula, CA, Feb. 5-10, 1992.
6. M. Sarikaya, J. Liu, and I. A. Aksay, "Characterization of Hierarchically Structured Natural Ceramic-Polymer Composites," 15th Asilomar Conference on Polymeric Materials, Org. by Eric Baer and Anne Hiltner, Pacific Grove, CA, Feb. 9-12, 1992.
7. I. A. Aksay and M. Sarikaya, "Processing of Nanocomposites by Biomimicking," The Minerals, Metals, and Materials Society and American Society of Materials, International, TMS/ASM Annual Meeting, San Diego, CA, March 1-5, 1992.
8. M. Sarikaya, "Introduction to Biomimicking and Structures of Biological Hard Tissues with Reference to Mollusca," March Meeting of American Physical Society, Indianapolis, IN, March 16-20, 1992.
9. M. Sarikaya, "Hierarchically Structured Natural Ceramic-Polymer Composites," Invitational Lecture Series, Allied-Signal, Inc., Morristown, NJ, June 25, 1992.
10. I. A. Aksay, "Bioinspired Processing of Ceramics and Composite Materials," Ceramics: Today and Tomorrow, Japan's 100th Anniversary Intl. Conf., Yokohama, Japan, Oct. 16-18, 1991.
11. I. A. Aksay, "Dispersion of Ceramic Nanosized Particles in Biological Systems," Plenary Lect., Engineering Foundation Conference, Palm Coast, FL, March 15-20, 1992.
12. I. A. Aksay, "Processing of Ceramics by Biomimicking," March Meeting of American Physical Society, Indianapolis, IN, March 16-20, 1992.
13. I. A. Aksay, "Trends in Processing of Ceramic-Polymer Composites with Colloids," 3M Technical Forum, St. Paul, MN, April 17, 1992.

14. M. Sarikaya, "Biomimetics: Design and Processing of Materials," Pfizer International, Morristown, NJ, September 2, 1992.
15. M. Sarikaya, "Nanodesign of Materials by Biomimetics," 1st International Conference on Nanocomposite Materials, Cancun, Mexico, September 22-25, 1992.
16. M. Sarikaya, "Nanodesigning of Materials by Biomimetics," 1st International Conference on Nanocomposite Materials, Cancun, Mexico, September, 22-25, 1992.
17. M. Sarikaya, "Materials Design by Biomimetics: Critical Issues," Tutorial Presentation, March Meeting of American Physical Society, Seattle, WA, March 15-19, 1993.
18. M. Sarikaya, "Biomimetics: An Introduction," National Technological University Broadcast, March 19, 1993 (Seattle, WA).
19. M. Sarikaya, "Biomimetics," Syracuse Univ., Syracuse, NY, April 5, 1993.
20. M. Sarikaya, "Biomimetic Design of Materials," National Institutes of Standard and Technology, Bathesda, MD, April 23, 1993.
21. M. Sarikaya, "Biomimetics," IBM, T. J. Watson Research Center, Yorktown Heights, NY, May 10, 1993.
22. M. Sarikaya, "An Introduction to Biomimetics," A Tutorial Lecture at 51th Annual Meeting of Microscopy Society of America, Cincinnati, OH, August 2, 1993.
23. M. Sarikaya, "Design and Processing of Materials by Biomimetics," AFOSR Workshop on Biomimetic Materials, Wright Laboratories, Wright-Patterson Air Force Base, Dayton, OH, August 12, 1993.
24. M. Sarikaya, "Biomimetics: Critical Issues," AT&T Bell Labs, Holmdel, NJ, 9/13/1993.
25. M. Sarikaya, "Growth And Shape Formation of Mollusc Shells: Hierarchical Morphogenesis," in Symposium S entitled Biomimetics, Materials Research Society Fall Meeting, Boston, MA, November 27 - Decemeber 3, 1993.
26. M. Sarikaya, Tutorial Lecture entitled: "Lessons from Biology: Structure-Property Relations in Hierarchically Structured Hard Biocomposites," Tutorial Lectures on Biomimetics, Materials Research Society Fall Meeting, Boston, MA, November 27 - Decemeber 3, 1993.
27. M. Sarikaya, Biomimetics: Current Issues and Future Prospects, Department of Materials Science and Engineering, University of Washington, Seattle, WA, January 10, 1994.
28. M. Sarikaya, Faculty of Science, Bilkent University, "Biomimetics: Rsearch Interests and Challenges," January 26, 1994.
29. M. Sarikaya, "Organic-Inorganic Interfaces in Layered Biological Composites," 16th Annual Asilomar Workshop on Advanced Polymers, Pacific Growe, CA, February 9-12, 1994.
30. M. Sarikaya, "Nanodesigning in Biological Composites," Spring Meeting of Materials Research Society, San Francisco, April 4-9, 1994.

31. M. Sarikaya, "Inorganic-Organic Interfaces in Biological Composites," in Workshop entitled: *Nanofabriaction and Biosystems: Frontiers and Challenges*, Kona, Hawaii, May 8-12, 1994.
31. M. Sarikaya, "Biomimetics," Gordon Conference entitled: *Model Membranes: From Biophysics to Materials Science*, Ventura, CA, Feb. 27 - March 4, 1994.
32. M. Sarikaya, "Organic-Inorganic Interfaces in Biocomposites," 52nd Annual Meeting of Microscopy Society of America, New Orleans, LA, August 5-9, 1994.
33. M. Sarikaya, Planary Lecture entitled: "Nanodesign and Properties of a Biocomposite for Biomimetics," 2nd Mexican Society of Electron Microscopy, Cancun, Mexico, September 26-28, 1994.
34. M. Sarikaya, "Structures and Properties of Biological Composites," ASME, Chicago, November 6-8, 1994.
35. M. Sarikaya, "Biomimeticization: From Biology to Technology," in Symposium S, entitled "Biomimetics and Biomolecularly Prepared Materials," Materials Research Society Fall Meeting, November 27 - December 2, 1994.
36. M. Sarikaya, "Biomimetics: Hard Tissues," Tutorial Lecture, Fall Meeting of Materials Research Society, Boston, MA, Nov. 27, 1994.

**b. Conference Presentations (contributed talks)**

A partial list of conference presentations is as follows:

1. J. Liu, M. Sarikaya, and I. A. Aksay, "Hierarchical Twin Structure in the Nacre of Red Abalone Shell," 49th Mtg. of Electron Microscopy Society of America, San Jose, CA, Aug. 4-9, 1991.
2. K. E. Gunnison, M. Sarikaya, and I. A. Aksay, "Structure-Mechanical Property Relationships in a Biological Ceramic-Polymer Composite," Materials Research Society Fall Meeting, Boston, MA, Dec. 2-6, 1991.
3. B. Flinn, M. Sarikaya, and I. A. Aksay, "Deformation Mechanisms in Biolaminates," 94th Annual Meeting of American Ceramic Society, Minneapolis, MN, April 12-16, 1992.
4. T. Ren, J. T. Staley, N. B. Pellerin, and I. A. Aksay, "Bacterial Polyglutamic Acid as an Aid in Ceramic Processing," 92nd Ann. Meeting of American Society of Microbiology, May 1992.
5. M. Sarikaya and I. A. Aksay, "Shape Formation in Biological Systems," MRS Fall Meeting, Nov. 28 - Dec. 4, 1992, Boston, MA.
6. M. Sarikaya and I. A. Aksay, "Shape Formation in Biological Systems," MRS Fall Meeting, Nov. 28 - Dec. 4, 1992, Boston, MA.

7. M. Sarikaya, "Biological Composites: Ultimate Self-Assembled Materials," XIIIth International Congress of Electron Microscopy, ICEM'94, Paris, July 17-22, France.
8. M. Sarikaya, "Organic-Inorganic Interfaces in Biological Composites," Proceedings of 52nd Microscopy Society of America, New Orleans, Aug. 5-9, 1994.
9. Daniel Frech and M. Sarikaya, "Formation, Crystallography, and Morphology of Biogenic and Geological Aragonite" Symposium S, entitled: Biomimetics ??, Materials Research Society Fall Meeting, Boston, MA, November 27 - December 2, 1994.
10. R. Humbert, G. Lancaster, C. Furlong, and **M. Sarikaya**, "Bioduplicated Pearls: Layered Aragonitic Particles Using Organic Shell Extracts" Symposium S, entitled: Biomimetics ?, Materials Research Society Fall Meeting, Boston, MA, November 27 - December 2, 1994.
11. Sima F. Hashemifar, **M. Sarikaya**, and J. T. Staley, "Environmental Aspects of Using Synthetic- and Bio-Polymers in Ceramic Processing," Symposium S, entitled: Biomimetics ??, Materials Research Society Fall Meeting, Boston, MA, November 27 - December 2, 1994.

### c. Organization of Symposia and Workshops

1. **Design and Processing of Materials by Biomimetics**, M. Sarikaya and I. A. Aksay, Workshop, held in Seattle, WA, April 1-3, 1991 (held during the continuation of the first 3-year portion of the current project; 12 papers by the invited speakers are being edited for publication as a book by American Institute of Physics (300 pages).
2. **Resolution in the Microscope**, Special Intl. Symposium, organized by M. Sarikaya, held during the 49th Annual Meeting of Electron Microscopy Society of America, San Jose, CA, Aug. 1-6, 1991, and the proceedings were published as a special issue of *Ultramicroscopy*, Vol. 47 [1-3] (1992) (24 papers; 300 pages)
3. **Hierarchically Structured Materials**, Symposium Z, organized by Ilhan A. Aksay, E. Baer, M. Sarikaya, and D. Tirrell, Fall Meeting of the Materials Research Society, Boston, MA, Dec. 1-6, 1991 (Papers in this symposium were published as MRS Proceedings, Vol. 255, 1992) (45 papers, 447 pages)
4. **Fundamental Materials Problems and Challenges in Biomimetics**, S-5, Organized by M. Sarikaya and e. A. Stern, Symposium of the Division of Condensed Physics, March Meeting of the American Physical Society, Indianapolis, IN, March 16-20, 1992.
5. **Microscopy of Self Assembled Materials and Biomimetics**, 49th Annual Meeting of Electron Microscopy Society of America, August 16-20, 1992, Boston, Mass. (Proceedings will be published as a special issue of *Journal of Microscopy Techniques and Research*, 1993).
6. **Determination of Nanoscale Physical Properties of Materials by Microscopy and Spectroscopy**, Proc. of MRS Symposium, edited by M. sarikaya, K. Wickramasinghe, and M. Isaacson, Vol. 332 (Materials Research Society, Pittsburgh, PA, 1994) (80 papers and 700 pages).
7. **Biomineralization: From Biology to Technology**, Symposium S, Materials Research Society Fall Meeting, 1995, Boston (planned).

### 4.3 Patents

Patent Application: "Process for Suspension of Ceramic or Metal Particles Using Biologically Produced Polymers," U. S. Patent Appl., Serial No. 071699.970.

### 4.4 News Clippings

(Some examples from popular science magazines and from the science sections of newspapers and newsmagazines)

1. "Mollusk Teaches Ceramics to Scientists," by Ivan Amato, *Science News*, Materials Science Section, December 9 (1989) p. 383.
2. Chemical Processing of Ceramics, by D. Ulrich, *C&EN*, January 1 (1990) pp. 28-40.
3. Nanostructured Materials, by Robert Cahn, in *News and Views* Section, *Nature*, 348 389-390 (1990).
4. "Biomimetics: Creating Materials From Nature's Blueprints," by Robin Eisner, *The Scientist*, July 8, 1991.
5. "The New Alchemy," Cover Story, *Business Weekly*, , July 29 (1991) pp. 48-55.
6. "Heeding the Call of the Wild," by Ivan Amato, *Science*, August 30 (1991).
7. "Natureworks: Making Minerals the Biological Way," by Elizabeth Pennisi, *Science News*, Feature Article, **141**, May 16 (1992) pp. 328-332 (Cover Story).
8. "Biomimetics," Cable News Network, National Broadcast in *Science and Technology* Program on December 15, 16, 19, and 20, 1992.
9. "Nature at the Patent Office," by Sharon Begley and Carolyn Friday, in Technology Section of *Newsweek*, December 14, 1992.
10. "Science Takes a Lesson from Nature, Imitating Abalone and Spider Silk," by Warren Leary, *The New York Times*, Science Section, Aug. 31, 1993
11. "The Mother of All Pearls," by D. Clery, in the Technology Section of *New Scientist*, 28 March 1992.
12. "Su licenza di Dio," In Section: *Technologia/I nuovi materiali*, Science Section in *PANORAMA*, 21 March(1993) pp. 153-154.
13. "Doing What Comes Naturally," by L. G. Blanchard, *Columns*, Sept. (1993) 20-23.
14. "Nature's Building Blocks," by T. Campbell, *POPULAR SCIENCE*, Oct. (1993) 74-77.
15. "Nature's Materials: Unlocking the Secrets of Charlotte's Web," by L. G. Blanchard, *IEEE Potentials*, (quarterly magazine) February 1994 (The Institute of Electrical and Electronics Engineers, Inc.) pp. 34-37.
16. "Biomimetics," in *Private Eye: Looking/Thinking by Analogy*, by Kerry Ruef (Seattle, 1993) (This is a book written for the elementary school students as a guide to developing the interdisciplinary mind, hands-on thinking skills, creativity, and scientific literacy. It has been adapted by many schools around Seattle).
17. *Curiosities: A Book of Scientific Trivia*, by Sharon McGrayne (John Wiley, New York, 1994).
18. *Sky Magazine*, Delta Airlines, April 1994.

## 4. Personnel

### Principal Investigators:

Mehmet Sarikaya (UW)  
 James T. Staley (UW) and  
 Ilhan A. Aksay (UW, now at PU; no contribution after September 1993)

### Research Scientists:

Daniel M. Dabbs (Materials Science and Engineering) (UW, now at PU)  
 (no contribution after 1993)  
 Jun Liu (Materials science and Engineering) (UW, now at Battelle Pacific  
 Northwest Laboratories, Richland, WA; no contribution after December 1992)  
 Nancy B. Pellerin (Microbiology) (UW; left in June 1994)  
 Brian Flinn (Materials science and Engineering) (UW, left in January 1993)  
 Maoxu Qian (Materials Science and Engineering) (UW; continuing)

### Graduate Students:

Tao Ren (Microbiology) (UW; Graduated in September 1994)  
 Hsien-Liang Kerr (Materials Science and Engineering)  
 (UW and PU; no longer in the program)  
 Katie Gunnison (Materials science and Engineering) (UW) (Graduated 1991)  
 Gordon L. Graff (Materials Science and Eng.) (UW) (Graduated 1991)  
 Daniel Frech (Materials Science and Engineering) (UW, continuing)  
 Benjamin Shapiro (Materials Science and Engineering, UW, continuing)  
 Sima F. Hasemifar (Materials Science and Engineering, UW; completed MS June  
 1994; started Ph.D. in Fall 1994).

### Undergraduate Students:

Jeffrey Frawley	(graduated) (Materials Science and Eng.) (UW)
Myeung Lee	(graduated) (Materials Science and Eng.) (UW)
Bill Wang	(graduated) (Chemical Eng.) (UW)
Susan Sawyer	(graduated) (Microbiology) (UW)
Sean Mallony	(graduated) (Microbiology) (UW)
Gretchen Wahl	(graduated) (Chemical Engineering) (UW)
Demetria Webster	(continuing) (Materials Science and Engineering)
Mary Katchur	(continuing) (Materials Science and Engineering)
Colleen Lasley	(continuing) (Chemical Engineering)